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PERMEABILITY

By WALTER STILES

CHAPTER XI

THE DETERMINATION OF THE PERMEABILITY OF PLANT CELLS TO DISSOLVED SUBSTANCES

THE passage of dissolved substances into living cells takes place for the most part independently of the absorption of water. Just as the latter enters the cell in order to bring about equilibrium on the two sides of the cell membranes, so the passage of a dissolved substance into the cell can only proceed until the distribution of the substance within and without the cell fulfils the conditions of equilibrium. Of course, if the passage of water into, or out from, the cell should alter the conditions of equilibrium with regard to dissolved substances, passage of dissolved substance across the cell membranes may take place, but there appears no reason for supposing there is any closer connexion between the entrance of water and solutes into plant cells.

The entrance of any solute into a cell depends therefore not only on the permeability of the cell membranes to the substance, but also on how far the distribution of the substance on the two sides of the membrane is removed from the equilibrium conditions. For this reason, although the passage of a substance into the cell can be taken as evidence of the permeability of the cell to the substance, the non-entrance of it need not necessarily indicate that the cell is impermeable to the substance. Similarly the rate at which a substance enters a cell cannot be taken as a measure of the permeability of the cell. If a dissolved substance were able to penetrate the protoplasm and combine with some constituent of the cell, it would obviously enter the cell to a much greater extent and at a greater rate, than if it did not combine with any constituent. In the latter case it might

even be difficult to discover whether the substance were permeable at all to living cells. For many substances which are harmless to cells when presented to them in low concentration have a toxic effect in stronger concentrations, and as a result of toxic action the permeability of the cell may be greatly increased. It may therefore often be necessary to use substances in dilute solutions if permeability of *living* cells is to be examined, and if no marked accumulation of the substance in the cell sap takes place its appearance there may be very difficult to recognise on account of the small quantity of it present. The necessity of distinguishing between intake or absorption of a substance by the cell and the permeability of the cell membranes to the substance has been rightly emphasized by recent writers (Höber and Nast, 1913; Brooks, 1917 *a*; Osterhout, 1917 *d*).

It may again be emphasized in this place that the cell is a very complex system with regard to its permeability. A dissolved substance in diffusing into the cell sap from the solution outside the cell has to pass through the cell wall and the protoplasm and possibly through plasmatic membranes on the inner and outer surfaces of the protoplasm which differ in permeability properties from the bulk of the protoplasm (cf. Stiles and Jørgensen, 1918). It is very generally impossible to distinguish between these different phases in attempting an analysis of permeability phenomena, so that in the following where the permeability of the cell or of the cell membranes is mentioned it means the permeability of the whole series of cell membranes which separate a liquid external to the cell from the cell sap in the vacuole.

It should be perfectly clear from what has been said of the permeability of membranes in Chapter V, that we may expect the permeability of the same cell to differ exceedingly towards different solutes. We may also expect that the order of permeability to a series of substances may differ in the case of different cells. Also as cells differ so much in their structure and properties we may expect that methods applicable for the determination of the permeability of some kinds of cells may not be applicable in the case of others. This is indeed the case, as will be very evident from a consideration of the various methods that have been evolved for the determination of permeability. These methods are described below.

I. DIRECT TEST OF PERMEABILITY BY OBSERVATION
OF VISIBLE CHANGES IN THE CELL

If a substance on entering the cell should produce a visible change in it, it is obvious that such visible change is evidence of the permeability of the cell to the substance.

(i) *Dyes.* The simplest application of this method is the investigation of the penetration of dyes into the cell, the permeability of which to a dye is shown by the coloration of the cell contents by the penetrating dye. Using this method Pfeffer (1886) examined the permeability of living cells of a considerable number of plant species to a number of dyes. He found that a passage of the dye into living cells took place with methylene blue[1], methyl violet, cyanin, Bismarck brown, fuchsin, safranin, methyl orange, tropæolin ooo, methyl green, iodine green, Hoffmann's violet, gentian violet and rosolic acid. These substances are taken up by the cell from very dilute solutions and accumulate in the cell sap so that the concentration inside the cell must be much higher than that of the external solution. Thus methylene blue diffuses into the root hairs of *Trianea bogotensis* and the outer cells of the root of *Lemna minor* among others, so as to give a deep blue colour to the cells even when the solution of the dye presented to the cells is as dilute as 0·0008 per cent. In some cases some of the dye does not accumulate in the vacuole in solution, but in the form of a blue precipitate as in the root hairs of *Azolla caroliniana* and in *Spirogyra communis*. When this is the case, continued immersion in the dye may lead to accumulation of the dye in solution in the cell sap in addition to the precipitate, as observed by Pfeffer in *Zygnema cruciatum*. The blue precipitate appears to be a compound with tannin, and accumulation of the dye in solution in the vacuole apparently only takes place when all the tannin present has combined with the dye. Pfeffer supposes the accumulation of the dye in solution in the vacuole is due to the formation of an undetermined soluble compound of methylene blue with some substance or substances present in the cell sap, and that the cell mem-

[1] In the English edition of Pfeffer's *Physiology of Plants*, 1 (1900), pp. 94, 96, 97, where these observations of Pfeffer are summarised, this dye is most unfortunately referred to as methyl blue, a dye which Pfeffer showed was incapable of penetrating into the cells he examined. In Pfeffer's original paper an unfortunate misprint occurs, methylene blue being included in the list of dyes which penetrate plant cells and also in the list of those which do not. The body of the paper makes it quite clear that in the latter case "methylen-blau" is a misprint for "methylblau."

branes are impermeable to the substance produced. There may, of
course, be a number of different substances present in the cell capable
of forming non-diffusible compounds with methylene blue or other
dyes, but the only substance in addition to tannin which has so
far been recognised as capable of this appears to be phloroglucinol
(Waage, 1890; Klemm, 1892). It might also be possible to account
for the accumulation of the dye in the cell sap by adsorption. If this
were so, one would expect the highly colloidal protoplasm to play
a greater part in adsorbing the dye than the less highly colloidal cell
sap. Nevertheless in the case of methylene blue no appreciable
coloration of the protoplasm by the dye is observable, but on the
other hand, rosolic acid was observed to enter the protoplasm so that
the latter became visibly coloured, whereas no accumulation of the
dye in the vacuole was observed. All the other dyes recorded above
as entering the cell both accumulate in the vacuole and stain the
protoplasm. How such "vital staining," that is, the staining of
living cells by the accumulation of dye ("intra-vitam stain") in the
cell, takes place, will be considered in a later chapter.

With nigrosin, aniline blue, methyl blue, marine blue, aniline grey,
eosin and congo-red, no visible intake of the dye into living cells
could be observed, although penetration may be rapid enough into
dead cells. Even after three days' immersion in a 1 per cent. solution
of aniline blue or a 0·5 per cent. solution of nigrosin no coloration was
observable of the cell sap of (?) root hairs of *Trianea bogotensis*
immersed in these very densely coloured solutions, and the cells were
uninjured. Similarly indigo-carmine when presented to *Spirogyra
setiformis* and *Trianea* in 0·7 per cent. solution, was not absorbed
even after four days; even the cell walls were not stained and the
cells remained alive.

Apparently none of the dyes examined by Pfeffer are absorbed
either by the nucleus or chromatophores if the cell remains alive.
Only after the death of the cell can the nucleus become stained,
and methylene blue, for example, can then be absorbed by the nu-
cleus. But Campbell (1888) working in Pfeffer's laboratory showed
a little later that the nucleus of living cells can be stained by some
dyes, as for example, dahlia and mauvein. Other cases have since
been recorded by Lauterborn (1893) and Palla (1893), for example.
This possibility was fully recognised by Pfeffer, who pointed out that
indigo-carmine had been shown to stain the nuclei of living kidney
cells (Heidenhain, 1874), while different kinds of cells may vary in
regard to the staining of bodies contained in them. Thus the micro-

somes in *Trianea* are usually intensively stained, while those of *Saprolegnia* and *Momordica* remain uncoloured.

This method has been much used to obtain information in regard to the mechanism of absorption and permeability, as, for example, by Overton (1899, 1900) who concluded that those dyes are absorbed by the cell which are soluble in lipoid substances, and Ruhland (1908 *a, b*, 1909 *a*) who at one time held that the uptake or non-uptake of a dye by living cells depends on whether the dye is basic or acid[1] respectively. This view was combated by Höber (1909) who had shown that a number of acid dyes were capable of staining the epithelial cells of the kidney. Ruhland supposed that the cell might be permeable to the acid dyes but that they combined with no cell constituent and so were not accumulated in the cell and consequently did not stain it sufficiently for the dye to be visible. The recent work of Collander (1921) indicates however that certain sulphonic acid dyes examined by him do not enter a variety of plant cells to any appreciable extent. Thus most cells placed in solutions of cyanol extra, orange G, methyl orange, Ponceau R and wool violet S for 24 hours or more became coloured with the dye to only $\frac{1}{8}$ to $\frac{1}{60}$ of the depth of colour of the external solution, while analysis of the external solution showed that this was the true explanation and that the dye was not absorbed and so not accumulated in the cell as a colourless compound of dye with a cell constituent.

Certain cells, however, were shown by Collander to be capable of accumulating these sulphonic acid dyes. Such are the cells surrounding the vascular bundles of the perianth leaves of a white flowering variety of *Hyacinthus orientalis*, and similar cells in a white flowered variety of *Tulipa Gesneriana*, a fact discovered by Rohde (1917).

A variant of the method was introduced by Küster (1911), who investigated the absorption of dyes from the cut surfaces of shoots or other organs immersed in a solution of dye. He found that a number of acid dyes were capable of passing into and staining cells when introduced to the cells in this manner. This conclusion was confirmed by Ruhland (1912 *a, b*) who also found that out of 30 basic dyes tested several were not absorbed by the bulb scales of the onion, namely, Victoria blue 4R and B, Basler blue R and BB, gallamin

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[1] A basic dye is one in which the radicle to which the colour is due is a base. Thus Bismarck brown is the colour base triamidoazobenzene

$$NH_2C_6H_4N:NC_6H_3(NH_2)_2;$$
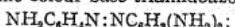
it is generally used as the hydrochloride. Similarly, in an acid dye the radicle to which the colour is due is an acid. Congo-red is such a case, in which the dye as used is generally the sodium salt of the acid.

blue and night blue, while two were absorbed only slowly, namely, diazin green and Victoria blue R.

The significance of these results in regard to the mechanism of cell permeability will be dealt with in a later chapter.

This method is obviously chiefly useful as affording a qualitative test of the penetration of dyes into plant cells, and although it furnishes indisputable evidence of the entrance of a penetrating dye, it is not safe to use it as evidence of the non-penetration of a dye when the cell sap remains uncoloured. Nor is the method a very suitable one for quantitative measurements. Nevertheless, Collander has made it so by mounting sections of tissue immersed for a certain time in the dye in solutions of the dye of different concentrations and so comparing the colour of the section with that of the surrounding solution. The solution the colour of which appeared of the same depth as that of the section is taken as having the same concentration as the dye in the cell sap. In this way Collander showed that after immersion in quite strong solutions of sulphonic acid dyes, the concentration of the cell sap in regard to dye is usually only a fraction of that of the external solution.

(ii) *Acids and alkalies.* If the cell should contain a substance which acts as an indicator for acid or alkali, the passage of substances belonging to one or other of these groups can be detected by the change in colour of the sap. De Vries (1871 *a, b*) observed the permeability of the cells of the root of red beet to ammonia by the change in colour of the pigment in the cells from red to blue.

Pfeffer (1877) extended this observation to other plant cells with coloured sap, such as the petals of flowers of *Pulmonaria* and the staminal hairs of *Tradescantia*, and showed that such cells are readily permeable to acids, including weak acids such as carbonic, acetic, tartaric and phosphoric acids, and to alkaline hydroxides as well as ammonia. If the reagent is used in dilute enough solution and the tissue well washed with water soon after treatment so that the acid or alkali is quickly removed, the original colour of the sap may return and the cell apparently suffer no injury by the treatment.

Haas (1916 *a*) found a number of tissues suitable for examining the permeability of acids and alkalies by this method, these tissues including the petals of *Browallia speciosa, Pelargonium* and *Hyacinthus orientalis*, var. *Queen of the Blues*, and the root of red radish. The rate of penetration of a number of acids and alkalies through these tissues was compared, the substances being used (1) in a concentration of 0·01 N, and (2) in such a concentration that the hydrogen-

ion or hydroxyl-ion concentration was 0·01 N. In the former series and with *Browallia* petals the order of alkalies was ammonium hydroxide, sodium hydroxide and potassium hydroxide, while the order of acids was salicylic, benzoic, trichloracetic, formic, hydrochloric, nitric, sulphuric, phosphoric, oxalic, tartaric (lactic, citric), acetic. The acids included in a bracket penetrated at the same very slow rate while the rate of penetration of acetic acid was extremely slow. The order of acids in the case of *Hyacinthus* perianth was much, but not quite, the same. In the second series, on the other hand, in which the acids were used with the same hydrogen-ion concentration, the order of penetration was quite different, acetic acid penetrating most rapidly of all the acids examined.

More recently Brenner (1918) has recorded observations made by this method, the tissue used being principally hypodermal cells of red cabbage and staminal hairs of *Zebrina pendula*. His conclusions are not identical with those of Pfeffer. Various acids, namely, hydrochloric, nitric, sulphuric, phosphoric, citric, malic, tartaric and oxalic acids, are stated to penetrate the undamaged protoplasm very slowly if they are presented in low non-toxic concentrations, while the somewhat higher concentrations, from which the acids enter more rapidly, injure the cell, which apparently accounts for the greater ease of penetration. The vitality of the cell after such treatment can be tested by plasmolysis and deplasmolysis, and, if the cells are suitable, by protoplasmic movement.

Bethe (1909), Warburg (1910) and Harvey (1911) have extended the method to colourless cells by first allowing the intra-vitam stain neutral red to penetrate the cells, which are thus stained red. Neutral red is an indicator, changing colour to yellow in alkaline medium. By this means Harvey showed the rapid penetration of ammonia and amines, whereas the strong bases diffused much less readily into living cells, and even their entrance might have been due to their toxic action rendering the cells more permeable.

(iii) *Salts other than dyes.* If a cell contains a substance with which a salt reacts to give either a precipitate or a different colour, the penetration of the salt into the cell can be observed by means of the formation of the precipitate or colour. Thus C. Darwin (1875) showed the penetration of ammonium carbonate into the root of *Euphorbia peplus* by the cloudy appearance produced in the cells. The aggregation produced by the same salt as well as by ammonium acetate and a number of other nitrogenous substances in the tentacles of *Drosera* was attributed by Darwin to the penetration of the salt or other

dissolved substance into the cells. Again if a cell contains soluble calcium salts, the entrance of a soluble carbonate or oxalate will result in the formation of a precipitate of the corresponding calcium salt. In this manner Pfeffer was able to show the permeability of cells to ammonium carbonate. Conversely if a cell contains soluble oxalates or carbonates, the permeability of the cell to calcium salts can be similarly determined. Osterhout (1910) showed that root hairs of very young seedlings of *Dianthus barbatus* formed crystals of calcium oxalate when the roots were in a solution of a calcium salt, but not otherwise. The following solutions were used: 0·005 M calcium sulphate, 0·005 M calcium nitrate, 0·005 M calcium chloride, 0·0001 M calcium hydrate, dilute artificial sea water and tap water. Crystals of calcium oxalate made their appearance in root hairs of *Dianthus barbatus* within four hours at 30° C. after immersion in the solutions, and in the case of calcium sulphate in 1·5 hours.

The penetration of iron salts into cells rich in tannin is rendered visible in the cell by the production of a blue colour. By the use of this test Miss Williams (1918 *b*) was able to show that hypodermal cells of the leaf stalks of London pride (*Saxifraga umbrosa*) are normally impermeable to ferric chloride, but that after treatment with various electrolytes the protoplasm becomes permeable to ferric chloride which enters the cells at such a rate as to give a definite blue colour within three minutes, whereas before treatment such a coloration is not produced after immersion for two hours in this salt.

The absorption of copper salts by a tree of *Quercus macrocarpa* was recognised on account of the metallic copper which accumulated in the cells (cf. MacDougal, 1899). Accumulation is made possible in this case by the reduction of the copper in the penetrating salt to metallic copper, so that equilibrium between the copper salt inside and outside the cell is not reached and endosmosis of the salt therefore continues.

(iv) *Sugars and Glycerol.* The penetration of glucose, sucrose and glycerol into the cells of water plants such as *Lemna*, *Elodea* and *Potamogeton*, can be demonstrated by the formation of starch from the absorbed sugars or glycerol inside the cells (Boehm, 1883; Meyer, 1886; Acton, 1890). The absorption of the sugar or glycerol takes place in the dark, while in control experiments with the same plants not provided with sugar or glycerol, no starch is formed.

Ruhland (1911) used this method to examine the permeability of the cells of sugar beet leaves to a variety of sugars. He found that raffinose, sucrose, maltose, glucose, fructose, certainly entered the

cells, as starch was formed in considerable quantities when the leaves were provided with these sugars in the dark. Galactose, mannose, sorbose, rhamnose and glycerol also entered the cells as indicated by the formation of starch, but no starch was formed in leaves provided with arabinose, xylose, erythrite, mannitol or dulcitol. However, this is not evidence of non-penetration of these substances, as the leaf cells may not be able to utilise them in the production of starch.

The value of similar evidence for the penetration of formaldehyde is slight (Spoehr, 1916; Jørgensen and Stiles, 1917).

(v) *Other substances.* A number of substances not included among the classes already mentioned will combine with some constituent of the cell sap to produce insoluble substances which thus become visible in the cell as precipitates. The penetration of the purine caffeine into cells was noted in this way by Pfeffer, the caffeine producing a precipitate with tannin contained in the cell. The permeability of antipyrin was observed in the same way by Pfeffer.

Similarly the penetration of substances producing colour in the cell can be observed. Thus Miss Williams (1918 a) has shown that living mycelia of *Penicillium glaucum* and *Oidium lactis* can absorb gold from colloidal solution, but that the gold is absorbed and retained by the cell walls which are stained blue.

2. DETERMINATION OF PERMEABILITY BY MEANS OF MICROCHEMICAL TESTS

The entrance of many substances into the cell can be made evident by treating the cell afterwards with some microchemical reagent which can be used as a test for the particular substance under examination. By this method Janse (1887 b) investigated the permeability of a number of plant cells to potassium nitrate. Following this substance the cells were treated with diphenylamine which gives a blue colour with nitrates (Molisch, 1883). Cells of *Chætomorpha aurea*, *Spirogyra nitida*, *S. crassa*, *S. communis*, *Tradescantia discolor*, *Curcuma rubricaulis* and *Stratiotes* were all found to be permeable to the inward passage of potassium nitrate. Wieler (1887) performed similar tests with seedlings of *Phaseolus multiflorus*, *Vicia Faba* and *Helianthus annuus*, testing in addition for potassium in the plants with platinic chloride with positive results. This method is obviously capable of wide extension as a qualitative test of permeability and absorption. It does not lend itself in its present form to quantitative measurements of either.

3. DETERMINATION OF PERMEABILITY BY ANALYSIS
OF THE EXPRESSED SAP OF CELLS AND TISSUES
OR OF CELL OR TISSUE EXTRACTS

Investigations of cell permeability by analysing the expressed sap of the plant material used after immersion in the experimental liquid have been made by a number of authors. The method is generally not applicable to single cells as only rarely can enough liquid be obtained from a single cell for the purposes of analysis, and in the case of cells forming part of tissues the individual cells cannot, of course, be separated from one another. Nevertheless, in two cases at least the method has been applied to single cells. Wodehouse (1917) made determinations of the permeability of single cells of the marine alga *Valonia* by this method. The "cells[1]" of this plant are so large that 1 to 2 c.c. of sap can be pressed out from cells of normal size, and up to 5 c.c. from large cells. Qualitative examination showed that potassium was much more abundant in the vacuole than in the surrounding medium (sea water), sodium and calcium were both present, but not more than a trace of magnesium although this occurs in some quantity in sea water. Chloride was present in high concentration in the cell sap, but sulphate was absent although it occurs in fairly high concentration in sea water. Conversely nitrate was found in the vacuole, whereas in sea water there was not enough to be detected by the qualitative tests employed[2]. The cell wall appears to play no part in the selective absorption thus demonstrated, for if living cells are killed and then replaced in sea water sulphates are soon present inside the cell.

The same method has recently been advocated for testing the penetration of dyes into living cells of *Nitella* (Irwin, 1922 *a, b*). The cells are so large that sufficient sap can be pressed out from a single cell for a colorimetric determination of the concentration of the dye that has penetrated into the cell. In this way it is held that quantitative investigation on the permeability of these cells to dyes is possible.

A method employed by Janse (1887 *b*) for testing the permeability of *Spirogyra* cells to potassium nitrate consists essentially in testing an extract of the cells with diphenylamine. Filaments of the alga, after immersion for various times in potassium nitrate solution, were made to burst in water containing diphenylamine. If the time of immersion of the *Spirogyra* in potassium nitrate solution had been

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[1] *Valonia* is a cœnocyte and the compartments are not regarded as true cells.
[2] These results have recently been confirmed by quantitative analysis (Osterhout, 1922 *a*).

sufficiently long a blue coloration was produced indicative of the fact that nitrate had diffused into the cells previously. The result of the test was negative if the time of immersion in the nitrate was short.

The analysis of tissues, expressed sap and extracts of tissues has been used considerably as a test of permeability. Thus Nathansohn (1903) made analyses of the expressed sap of the marine alga *Codium* after immersion in a solution of sodium nitrate and was in this way able to obtain data with regard to the penetration of sodium and nitrate ions into this plant. Paine (1911) used the same method to investigate the permeability of yeast cells to inorganic salts, and concluded that the slight intake he observed was entirely due to adsorption of the salt by the cell wall.

Analyses of whole plants growing in soil, water culture and other media have afforded much information with regard to the relative intake of different substances by living normal plants. The long series of ash analyses of various plants made by Wolff (1864–1880) and others has shown that compounds of potassium, sodium, calcium, magnesium and iron, and compounds containing chlorine, sulphur, phosphorus and silicon are present in considerable quantity in practically all plants examined, and it must be therefore concluded that such compounds are capable of penetrating into the cells of plants. Aluminium and manganese also appear to be regular constituents of plant ash, though often in very small amount, so it must be supposed that compounds containing these substances are capable of penetrating through the cell membranes of plants. The relative quantity of these various ash constituents varies however in different species (cf. for example, Grandeau and Bouton, 1877), and the differences cannot be correlated with differences in composition of the medium from which the plant obtains its ash constituents, although plants of the same species can take in more of any particular constituent from a medium which is rich in the constituent than from a medium poor in it (cf. Malaguti and Durocher, 1858, for the case of calcium).

Apart from the work of Salm-Horstmar (1851, 1856) with soil cultures and Sachs (1860, 1861) and Knop (1860) with water cultures, directed to determine the elements essential for plant nutrition, the work of Mazé (1913 *a, b*, 1914), Monnier (1905), Déléano (1907, 1908 *a, b*) and Pouget and Chouchak (1913) deserves mention in this place, as these authors have used the method of chemical analysis to determine the intake of different mineral constituents at different stages in the development of the plant. Their results were obtained, however, from the point of view of the part played by inorganic salts

in the development of the plant, and have only a passing interest from the point of view of permeability.

The chemical analysis of the whole plant can, of course, also be employed as a test of the permeability of the cells to substances other than salts. Thus Bourget (1899) showed that members of the Liliaceæ and Chenopodiaceæ were able to absorb considerable quantities of iodine through their roots, while the cells of the roots of potato were apparently impermeable to this element. Similarly, it is well known that some marine algæ absorb iodine from sea water to such an extent that they have been used as a commercial source of that element. By direct analysis Stoklasa, Šebor, Týmich and Cwacha (1922) have shown that aluminium ions are absorbed by the hydrophytes *Gale-opsis versicolor* and *Caltha palustris* and the mesophytes *Dactylis glomerata* and *Festuca pratensis*, but scarcely by the xerophytes *Sesleria cærulea* and *Anthyllis Vulneraria*.

Brooks (1917 *a*) raises the objection to this method that the quantity of substance found by analysis of tissue extracts includes any of the substance held in the cell walls and intercellular spaces. It is clear that the method cannot be used as a method for the quantitative measurement of *permeability* of the cell membranes, as other factors, namely, adsorption and chemical action, may influence the quantity and rate of absorption of the substance. When expressed sap is analysed there is the additional objection that it is difficult to obtain a reliable sample of sap, although this difficulty can apparently be overcome by suitable methods of extraction (cf. Chapter IX).

4. DETERMINATION OF PERMEABILITY BY VISIBLE CHANGES IN THE EXTERNAL MEDIUM

Visible changes in the external solution can be used in some cases as a test of the permeability of the cell membranes to substances contained in the cell. Thus when the cell sap contains a pigment the diffusion of this out of the cell can be observed by the coloration of the external liquid as soon as sufficient of the pigment has exuded, and the quantity that has passed out from the cells or tissues employed can be estimated colorimetrically (cf. Stiles and Jørgensen, 1917 *a*). If the external solution should contain an indicator, the diffusion out of acids or alkalies can similarly be observed, while if any of the constituents of the cell sap should give a precipitate or a colour reaction with a substance present in the external liquid, the exosmosis of this constituent can be similarly observed. The disad-

vantage of this method of studying exosmosis is that the quantities of diffusing substances may be too small for determination.

The change in colour of a solution of a dye external to the cells or tissue can similarly be used to determine the absorption of the dye, and by colorimetric estimation the method may be made quantitative. The chief objection that can be levelled against this means of determining intake is that a substance diffusing out from the tissue might react with the dye to produce a colourless or differently coloured substance, so that a decrease in the intensity of the colour of the external solution might not be due actually to an absorption of the dye by the tissue. That this may happen in some cases appears to be suggested by an observation of Miss Redfern (1922 *b*) to the effect that a solution of congo-red *deepens* in colour after disks of carrot root are immersed in it for some time. As such a deepening of colour does not occur in a solution of the dye to which no tissue has been added, the only possible explanation appears to be that the colour is altered by the exosmosis of some substance from the tissue. A more serious difficulty arises from the fact that the dye may be absorbed by the cell walls and never reach the protoplasm or vacuole. While this appears to be the case sometimes, it is not always so, and clearly observations by this method are free from objection if supported by others following the first method described in this chapter.

5. DETERMINATION OF PERMEABILITY BY CHEMICAL ANALYSIS OF THE MEDIUM EXTERNAL TO THE TISSUE

This method is similar to the one previously described, but as a quantitative method of determining absorption it is as a rule more refined. It has found considerable use as a method of investigation of cell permeability and intake or excretion. A decrease in the concentration of a substance in the external solution indicates absorption of the substance by the cells or tissue while increase in its concentration indicates exosmosis. As a qualitative test of permeability and a quantitative measurement of absorption and excretion the method is open to the same objections as that just described. It cannot be used quantitatively for measuring permeability any more than other methods described previously in this chapter.

Information relating to absorption of salts has been obtained by this method by a number of workers, both entire plants and pieces of tissue having been used. Among those who have worked with the former may be mentioned particularly Demoussy (1900) who deter-

mined the relative absorption of potassium and calcium by wheat and maize, Colin and de Rufz de Lavison (1910 *a*, *b*) who investigated the absorption of barium, strontium and calcium, Pantanelli (1915 *a*, *b*, *c*, 1918) and Pantanelli and Sella (1909) who paid particular attention to the unequal absorption by a number of species of the two ions of the same salt, and Miss Redfern (1922 *a*) who investigated the same problem critically in the case of the absorption of calcium chloride by the edible pea and maize. The absorption of glucose by the roots of the latter plant was shown by Laurent (1897).

Absorption of salts by disks of storage tissue was examined by Nathansohn (1903, 1904 *a*, *b*), Meurer (1909) and by Ruhland (1909 *b*). The results obtained by these various workers will be dealt with in the next chapter.

The exosmosis of any particular substance can, of course, be examined by this method. Thus it has been shown that sugars, chiefly sucrose, can diffuse out of the cells of the leaf of the sugar beet when they are immersed in water (Puriewitsch, 1898; Ruhland, 1911).

6. DETERMINATION OF PERMEABILITY BY MEASUREMENT OF THE ELECTRICAL CONDUCTIVITY OF THE EXTERNAL SOLUTION

The exosmosis of electrolytes from plant tissue into a surrounding medium consisting of pure water or a solution of a non-electrolyte will result in an increase in the electrical conductivity of the external solution. Measurement of the electrical conductivity of the latter can therefore be used as a criterion of the permeability of the cell membranes to the electrolytes of the cell. The method cannot of course be used to determine the exosmosis of any particular electrolyte, but it can be used for approximate quantitative determinations of exosmosis by assuming the electrical conductivity of the external solution to be a measure of the concentration of electrolytes in the solution. This is, of course, only approximately true, since the mobilities of different ions are different and with increasing concentration of the electrolytes the degree of dissociation will decrease, and the electrical conductivity is dependent on the mobilities of the ions and the degree of dissociation. However, with the exception of hydrogen and hydroxyl ions, the mobilities of ions likely to diffuse out of plant cells do not differ greatly among themselves, while the degree of dissociation will not be depressed greatly in the dilute solutions likely to be involved. If this external solution contains a non-electrolyte the presence of this will depress the conductivity,

and in comparative experiments allowance may have to be made for this (Stiles and Jørgensen, 1917 a).

Using this method Briggs and True and True and Bartlett (1912) showed the excretion of salts into distilled water by the roots of seedlings of *Lupinus albus* and field peas, while Stiles and Jørgensen (1917 a) investigated the influence of a number of organic substances on the exosmosis of electrolytes from potato tuber tissue.

The method is also usable within limits to determine the intake of electrolytes by plant tissue. Here a complication arises because intake of the salt by the tissue and excretion of electrolytes by the tissue will influence the conductivity in different directions. Interaction of the exudate and the constituents of the external solution may also be a disturbing factor. Results obtained may thus be sometimes very difficult to interpret (Stiles and Jørgensen, 1915 a). Nevertheless, by allowing for the exosmosis it may be possible to obtain quantitative results as has been done in regard to the absorption of a number of salts by carrot root by Stiles and Kidd (1919 a, b), who have pointed out the causes making for a fall or rise in the conductivity in the external solution, and have shown that in the results published by them the values obtained can be taken with confidence as minimum values for absorption. These results will also be dealt with in the following chapter.

7. DETERMINATION OF PERMEABILITY AND ABSORPTION BY MEANS OF ELECTROMETRIC MEASUREMENTS WITH THE HYDROGEN ELECTRODE

The exosmosis of acids and alkalies can be determined by measuring the concentration of the hydrogen ions in the external solution by means of the hydrogen electrode. A description of the method and the various forms of electrodes that have been devised will not be entered into here; those interested should consult the recent work of Clark (1920) in which the measurement of hydrogen ions is adequately treated. Similarly the absorption of hydrogen or hydroxyl ions from an acid or alkaline solution can be determined. The method has been used for measuring the absorption of hydrogen ions from dilute solutions of hydrochloric acid by Stiles and Jørgensen (1915 b), and of a number of other acids by Miss Hind (1916).

8. DETERMINATION OF PERMEABILITY AND ABSORPTION BY COLORI-
   METRIC ESTIMATION OF THE HYDROGEN ION CONCENTRATION
                   OF THE EXTERNAL SOLUTION

Changes in the concentration of hydrogen ions in the external
solution can also be determined colorimetrically by the indicator
method (see Clark, 1920). The scope of the method is exactly the
same as the preceding, although the results obtainable are not so
accurate. Using this method Haas (1916 b) has shown that no excre-
tion of acid apart from carbonic acid takes place from the roots of
maize and wheat dipping into distilled water. No excretion of alkali
occurs in the case of maize, while with wheat there is only a very
slight increase in the alkalinity of the water after the roots had
decayed.

9. TEST OF PERMEABILITY BY METABOLIC ACTION

While ash analyses have shown what substances are normally
absorbed by plants, growing plants on artificial media of known com-
position has shown which elements are necessary for normal growth.
If such an element is supplied to a plant in only one compound and
the plant grows healthily so that its metabolism is normal, it follows
that that substance must be able to penetrate into the plant. Thus
it has been shown that although nitrogen is most suitably provided
to most higher plants in the form of nitrates, in many cases other
nitrogen compounds are readily absorbed. Thus ammonium salts,
urea, various amino-acids such as glycine, asparagine, leucine and
tyrosine, uric and hippuric acids, acetamide and propylamine are
among nitrogen compounds that have been shown to be absorbed by
various species by means of the continued growth of the plants when
supplied with no other source of nitrogen. The literature dealing with
the possible sources of nitrogen for Fungi and Bacteria is enormous
and cannot be entered into here.

The penetration of inorganic salts through the leaves of *Thunbergia*
and other plants was shown in this way by supplying the roots with
nothing but water and spraying the leaves with a solution of nutrient
salts, or by immersing them in the solution (Dandeno, 1901).

The healthy growth of algæ such as *Nostoc* (Bouilhac, 1897) and
Stichococcus (Artari, 1901; Matruchot and Molliard, 1902) in the dark
on solutions containing glucose or other organic sources of carbon
indicates the permeability of the cell membranes to the sugar or
other organic nutrient.

In a similar way the unhealthy development or the death of a plant may be taken as evidence of the penetration into it of a poisonous substance if this is present in the external medium in addition to all the necessary nutrients. The test is equally legitimate if the presence of a dispensable substance in the external medium should have a stimulating effect on the plant and bring about a development above the normal. Thus the penetration of copper salts into roots of cereals and other plants has been made abundantly clear from the work of Phillips (1821) and many later writers, particularly Otto (1893), Coupin (1898), Kahlenberg and True (1896), Copeland and Kahlenberg (1900), True and Gies (1903) and Miss Brenchley (1910) among others. In a similar way the absorption of compounds of zinc, arsenic, manganese and boron has been shown, for a review of the work on which the reader is referred to the paper and book of Miss Brenchley (1914 *a*, *b*) dealing with the effect of these substances on plant growth. In Miss Brenchley's book will be found abundant references to the literature of the subject. Even compounds of the alkali metals (Coupin, 1901) have been shown to penetrate the roots of cereals by their harmful effect on vegetative development. The penetration of cyanides has been indicated in the same way (Brenchley, 1917).

Since this method can only afford a qualitative test of permeability, and has done so only incidentally, it is not worth while to deal with it in any greater detail here. It must, however, be pointed out, that the toxicity of a substance may indeed be a clear indication that it *reaches* the protoplasm, but it is no indication that the substance has the power of *passing through* the protoplasm before the properties of the latter, in respect of permeability, have been completely changed by the action of the substance. The test of toxicity is perhaps the least useful of all the tests of permeability.

10. PLASMOLYTIC METHODS

Concentration required to produce plasmolysis. It has already been pointed out in Chapter IX that a solution of a substance incapable of penetrating into the cell which just does (or just does not) produce plasmolysis, is, on the simple osmotic view of the cell, isotonic with the cell sap at the moment when plasmolysis is just about to commence. It must be pointed out, however, that if the sustance penetrates the cell sap but is immediately withdrawn from solution to form an insoluble compound, plasmolysis may still occur if the quantity of salt withdrawn from the external solution is insufficient to alter materially the concentration of the external solution. It is not

even necessary that an insoluble compound should be formed so long as the new combination of substances in the vacuole does not exert any higher osmotic pressure than previously. That such possibilities are by no means to be ignored is made very clear from the work of Pfeffer on the penetration of dyes described earlier in this chapter. Similarly, Stiles and Jørgensen (1917 *b*) have shown that storage tissues actually plasmolyse in solutions of common salt and remain so for 20 hours or more, while Stiles and Kidd (1919 *a*, *b*) have shown that sodium chloride is actually absorbed with some rapidity by such tissues.

But if such complication does not enter into the case, the penetration of a substance into the cell will raise the osmotic concentration of the cell sap, and hence the external solution will no longer be able to effect plasmolysis. In order to bring this about a higher concentration of the solution will be necessary, and even then, if the entrance of solute continues the cell will recover from plasmolysis.

If then the osmotic concentration of a substance required to produce plasmolysis is higher than that required in the case of a substance which is known not to penetrate the cell, it may be concluded that the former substance penetrates the cell.

Sucrose is generally chosen as a substance which penetrates the cell extremely slowly or not at all, and it is fortunate that the values of the osmotic pressure of this substance are more firmly established than those of other substances. The concentrations of other substances isotonic with sucrose solutions have been determined chiefly by indirect methods, such as calculations from the freezing point lowering, or from determinations of the electrical conductivity, as very few direct determinations of osmotic pressure of substances other than sucrose have been made (cf. Chapter VI). If the isotonic coefficient found for a substance by the plasmolytic method is lower than that found by direct measurement or by calculation from other physicochemical data, it is concluded that the cells used for the determination of the coefficient are permeable to the substance examined.

In this way de Vries (1888 *a*, *b*, *c*) showed that to produce plasmolysis of the cells of *Spirogyra nitida* a solution of glycerol was necessary of considerably higher concentration than that isotonic with a solution of sucrose which would produce plasmolysis. This method was later extended to a large number of different cells, and it was found that glycerol is very generally permeable to plant cells, although there are exceptions. Thus de Vries showed that the cells of the bud scales of *Begonia manicata* are impermeable to glycerol and also to

urea (de Vries, 1889 *a*, *b*). Later Overton (1895) tested the plasmo-
lytic action of a large number of substances, and with many was
unable to bring about plasmolysis at all, notably with the alcohols
of the fatty series, various narcotics (ether, chloral hydrate) and other
organic substances.

"*Permeability Coefficients.*" By determinations of the concentra-
tion of penetrating substances required to produce plasmolysis and
the concentrations of a non-penetrating substance required to pro-
duce plasmolysis of the same cells, Lepeschkin (1908 *a*) and Tröndle
(1909, 1910) claim to be able to obtain values termed "permeability
factors" by the former and "permeability coefficients" by the latter,
which are measures of the permeability of the cell. Thus let it be
supposed that the osmotic pressure of a non-penetrating substance
that just brings about plasmolysis is P_0 while the osmotic pressure
of the penetrating substance required to effect plasmolysis is P. The
osmotic pressure of the penetrating substance isotonic with the solu-
tion of non-penetrating substance is also P_0. As P is greater than P_0,
it follows that the isotonic coefficient of the penetrating substance
determined by plasmolysis will be less than that obtained by direct
measurements of the osmotic pressure with a perfectly semi-per-
meable membrane, or that calculated from determinations of the
osmotic pressure from other physico-chemical data.

If i is the true isotonic coefficient calculated from physico-
chemical data and i' is the isotonic coefficient obtained by the plasmo-
lytic method with the particular cells and substance under investiga-
tion, the permeability factor or permeability coefficient is given by

$$\mu = \mathrm{I} - \frac{i'}{i}$$

and this permeability factor or coefficient is held to be proportional
to the permeability of the cells employed to the particular substance.

It is difficult to understand how such considerations can lead to
results having any definite quantitative significance. We have ob-
served that the reason why a higher osmotic concentration of a sub-
stance that penetrates is required to effect plasmolysis than of one
which does not, is because some of the dissolved substance immedi-
ately enters the cell and so increases the osmotic concentration of
the cell sap. The difference between the osmotic concentration of a
penetrating and a non-penetrating substance required to bring about
incipient plasmolysis is therefore a measure of the amount of sub-
stance which has entered the cell between immersion in the solution
of the penetrating substance and the moment of observation. The

entrance of substance commences the instant the cells are placed in the solution but no doubt takes some time, and probably some very considerable time, before equilibrium is attained. The apparent isotonic coefficient will therefore vary with the time that has elapsed before the plasmolysed cells are observed. This was indeed recognised by Tröndle, but he appears to regard the lowering of the apparent isotonic coefficient which must result with increase in the osmotic concentration of the cell as indicating an increase in permeability. If the opinions of Lepeschkin and Tröndle have been interpreted aright, the so-called permeability factors or coefficients could only be used as quantitative measures of intake if the apparent isotonic coefficients were determined after a definite and constant time of immersion of the cells in the solution. Even then exosmosis is neglected, and it must be assumed that the substance does not react with a cell constituent. Defining the permeability of the cell to dissolved substance as the quantity of the substance diffusing through unit area of the cell membranes in unit time when there is unit difference of concentration of the substance between the external solution and the cell sap, it is clear that the method of permeability coefficients could not be used without modification and elaboration to give measures of the permeability of cells to dissolved substances.

Recovery from plasmolysis. If a cell is plasmolysed by a hypertonic solution of a penetrating substance, the entrance of the substance into the cell, provided no complicating factors are present such as removal of the substance into an osmotically inactive state, or excessive exosmosis, will increase the osmotic concentration of the cell sap, and in consequence the cell will slowly recover from plasmolysis. Recovery from plasmolysis can thus be used as a test of permeability, and has indeed been one of the favourite methods of investigating permeability since it was first introduced.

This method appears to have been first used by de Vries (1871 *a*) who showed the permeability of beet root cells to sodium chloride by its means. Later Klebs (1887, 1888) recorded the permeability of cells of *Zygnema* to glycerol on account of the recovery of cells of this alga from plasmolysis with a 10 to 20 per cent. solution of this substance. Since then a number of attempts have been made to obtain quantitative data with regard to the entrance of salts and the permeability of cells by means of plasmolytic data, and various methods have been devised which will now be described.

Tröndle's Method. Tröndle (1920) has attempted to adapt this method to the measurement of the quantity of a substance absorbed

in a certain time. Pieces of tissue assumed to be similar are placed in solutions of the substance of various concentrations, and the tissue examined at different times from the commencement of the experiment. The concentration of the substance in which plasmolysis is just visible is noted at each time. Then if after the lapse of 10 minutes this concentration is xN and after the lapse of a further 10 minutes yN, it is assumed that a quantity of salt has entered the cells of the tissue sufficient to raise the concentration of the substance in the cell sap by a quantity $(y - x)N$. This method, like other work of the same author, assumes that the external concentration of the substance has no influence on the rate at which it is absorbed, which indeed Tröndle emphasizes is the case in salt absorption, an assumption the evidence in favour of which is very doubtful, and the evidence against which is very strong.

Lepeschkin's Method. An attempt to obtain data with regard to the entrance of glycerol into cells of *Spirogyra* was made by Lepeschkin (1908 *a*) in the following way. Cells were plasmolysed in a solution of sucrose, which is assumed not to enter the cells, and left in the solution for an hour, when their volume was measured. The sugar solution was then replaced by a solution of glycerol and the volume again measured after 0·5 hour and again after a further two hours in the glycerol solution. Then if the volume of the cell after the first 0·5 hour is V_2 and after a further two hours V_3, the quantity of glycerol which enters the cell in two hours is

$$\frac{C\,(V_3 - V_2)}{1000}\ \text{gm.-mols.},$$

where C is the concentration of the glycerol solution in gm.-mols. and the volume is measured in c.c.

Lepeschkin defines the permeability as the ratio of the number of gram-molecules of the substance which pass through unit area of the cell membrane in an hour to the difference in concentration of the substance on the two sides of the membrane. If then the mean surface of the cells is S, the permeability is

$$\frac{V_3 - V_2}{1000\,S\left[1 - \dfrac{(V_3 - V_2)\,(V_3 + 4V_2)}{8V_2V_3}\right]},$$

for the mean difference in concentration between the external and internal concentrations of the substance (glycerol) is

$$C - \frac{1}{2}\left[\frac{(V_3 - V_2)\,C}{4V_2} + \frac{(V_3 - V_2)\,C}{V_3}\right],$$

that is
$$C\left[1 - \frac{(V_3 - V_2)(V_3 + V_2)}{8V_2 V_3}\right].$$

In this way Lepeschkin found values for the rate of entrance into the cells varying between 67×10^{-9} and 183×10^{-9} gram-molecules of glycerol per sq. cm. per hour in the case of glycerol penetrating into *Spirogyra*. These numbers are taken as direct measures of the permeability.

Values of the "permeability factor" were also obtained from calculations of the apparent isotonic coefficient of glycerol made at the same time on the same cells as those used for direct determinations of the "permeability."

This was done in the following way. It will be recalled that the cells were first plasmolysed in sucrose (of concentration C_0) and the volume of the cells measured after an hour. This volume is V_1. The cells were then transferred to a solution of glycerol of concentration C and the volume measured again after the lapse of 0.5 hour and again after the lapse of a further two hours, the two volumes being respectively V_2 and V_3. Since the cell is increasing in volume at the rate of

$$\frac{V_3 - V_2}{2}$$

per hour, its volume immediately after addition of the glycerol should have been

$$V_2 - \frac{V_3 - V_2}{4}.$$

Hence the concentration of glycerol apparently isotonic with the sucrose used is

$$C_x = \frac{V_2 - \dfrac{V_3 - V_2}{4}}{V_1} C$$

and the apparent isotonic coefficient of glycerol is

$$1.88 \frac{C_0}{C_x},$$

since 1.88 is the isotonic coefficient of sucrose.

Values of the "permeability factor" calculated in this way were found to be roughly proportional to the actual determinations of the permeability. Considering the very doubtful value of "permeability factors" it is surprising that the agreement should be as close as that actually found.

Fitting's Method. Fitting (1915) has devised a method for estimating the intake of salts by the rate of deplasmolysis, using cells of

Tradescantia (Rhœo) discolor. Similar cells which may be supposed to have the same osmotic concentration were plasmolysed in solutions of a salt of different concentrations. If the salt enters the cells, the latter deplasmolyse. Now a stronger solution will produce a greater degree of plasmolysis than a weaker solution, so that as cells that have been plasmolysed in the stronger solution deplasmolyse, a stage will be reached in which the degree of plasmolysis is the same as that originally produced in the weaker solution. During the time that elapses between the commencement of deplasmolysis and the reaching this less degree of plasmolysis, a quantity of salt must have entered the cells sufficient to increase the concentration of the salt in the cells by the difference in concentration between the weaker and stronger solutions. The degree of plasmolysis is measured by a rough estimate of the proportion of cells in the preparation which are plasmolysed, as, for example, one-half, or three-quarters. As there is no great precision in this mode of determining the degree of plasmolysis, Fitting's method cannot be regarded as an exact one. It also does not take account of exosmosis from the cells, which Fitting supposes is eliminated or rendered negligible by a preliminary treatment with water, a conclusion which is probably not correct. Results obtained by its means by Fitting and Tröndle will be dealt with in the next chapter.

Höfler's plasmometric method. The principle of the method used by Höfler (1918 *a*, *b*, 1919) is the same as that of the preceding, but the degree of plasmolysis is determined by actual measurements of the cells examined, so that the rate of deplasmolysis is followed in individual cells. The cell is plasmolysed by a decidedly hypertonic solution, the degree of plasmolysis being p_1. After the lapse of a time t the degree of plasmolysis is p_2. Then if the concentration of the external solution is C, and the osmotic concentrations of the cell corresponding to the two degrees of plasmolysis are respectively C_1 and C_2, we have

$$C_1 = Cp_1,$$

and

$$C_2 = Cp_2,$$

whence

$$C_2 - C_1 = C(p_2 - p_1).$$

Since the change in osmotic concentration of the cell in unit time is a measure of the rate of intake of the dissolved substance, the rate of intake can be determined from the concentration of the external solution and the change in the degree of plasmolysis.

Höfler's method, like those of Lepeschkin and Fitting, neglects the possible effects of exosmosis. Apart from this and the assumption

that the substance diffuses into the cells and there undergoes no reaction with the cell contents, the method seems to be a sound one for the determination of the absorption of dissolved substances. Höfler, however, like so many other observers, confuses the rate of intake with permeability. Thus, he defines permeability as the quantity of substance entering the cell in unit time, thus neglecting the fact that the rate at which the substance enters the cell depends not only on the permeability of the cell membrane, but also on the difference in concentration of the substance in the external solution and in the cell sap. This does not matter so much, as Höfler makes it perfectly clear what he is measuring, so that there is no obscurity. His results will be dealt with later.

11. The Determination of Permeability by Changes in Weight or Volume of Turgid Tissues

The principle of this method is much the same as that of the plasmolytic method. If turgid tissues are immersed in a solution of a substance strong enough to produce a contraction in the volume of the cells, but not concentrated enough to produce plasmolysis, the penetration of the dissolved substance into the cells of the tissue will bring about a gradual increase in volume (and consequently in weight) of the tissues. This method has been employed to test the entrance of salts and other substances into animal tissues and cells, *e.g.* blood corpuscles, by Koeppe (1895 *a*, *b*), Kozawa (1913, 1914) and others. For work with plant tissues the method has been developed by Lundegårdh (1911) who used it to study the entrance of salts into roots of *Vicia Faba*. For this purpose the roots were placed horizontally in a small cell under the microscope and changes in length of the root measured. The roots were first treated with a solution of the salt under investigation which produced a contraction in length. As the salt entered the tissue the root gradually increased in length. The time taken for the root to increase in length from 25 per cent. to 75 per cent. of the total increase it underwent was taken as a rough measure of the rate of entrance of the salts.

12. Tissue Tension Method

A tissue tension method for studying permeability to dissolved substances has been described by Brooks (1916 *c*). The experimental procedure is as follows. Similar strips of the peduncle or midrib of dandelion (*Taraxacum officinale*, Weber) were fixed at one end between

the two halves of a partially split rubber stopper and the strips held
horizontally so that the free ends could move in a horizontal plane. The
strips so held were immersed in 20 c.c. of a solution of the substance
to be examined in which the strips underwent no appreciable change
in curvature. The concentration of the solution was then increased
by the addition of a known small volume of a molecular solution of
the salt. As a result the strip of tissue underwent a decrease in
curvature, which soon ceased and was followed by a slow increase in
curvature. The time that elapsed between the increase in concentra-
tion and the instant that the strip regained its original curvature was
called the time of recovery. Immediately on recovery the concentra-
tion of the solution was again raised and the time of recovery again
noted. The process was repeated several times and in this way a series
of times of recovery was obtained. An empirical value for the rate
of penetration was obtained by dividing the change in concentration
by the time of recovery. Curves were then plotted between these
rates of penetration and the times that had elapsed between the first
immersion of the tissue in the solution and the middle of each recovery
time. These curves are supposed to represent the rate of penetration
of the salt into the tissue.

Brooks appears to regard the values he obtains for rate of pene-
tration as measures of permeability. He has good grounds for doing
this, as the difference in concentration between external solution and
cell sap with regard to the substance under investigation is the same at
the moment at which each recovery period commences. But the
effects of exosmosis are not considered, nor are the possibilities of
any reactions between the penetrating substance and the contents
of the cell sap. Also the results obtained during the first 15 or 20
minutes were very irregular, and were therefore disregarded. The
method in its present form is thus not free from objection.

13. DIFFUSION METHOD

This method has also been described by Brooks (1916 a, 1917 b).
Disks cut from the thallus of *Laminaria Agardhii* were placed between
two short pieces of glass tube applied to the two sides of the tissue
so as to make a watertight join. The lower piece of tube was closed
below by a rubber tube and clip. The cell thus formed was filled with
a salt solution having the same conductivity as sea water, or with
sea water itself, and the upper tube with a known quantity of a solu-
tion of the same composition but of one-half the concentration. The

rate of increase of the electrical conductivity of the solution in the upper tube and the decrease in the conductivity of the solution in the lower tube was taken as a measure of the rate at which the salt or salts in the lower cell passed through the tissues, and therefore as a measure of the permeability of the tissue. To eliminate errors due to exosmosis from the cells and diffusion from the intercellular spaces of the tissue, control experiments were made in which the more dilute solution was contained in both upper and lower tubes. The average conductance of the solution in the upper tube of the controls at the end of the experiments was taken as the standard, and the average conductance of the solutions in the experimental tubes "divided by this figure in order to obtain the percentage which expresses their gain as compared with the control." By this method it is stated that "the figures which were obtained in this manner measure the amount of salt which had passed through the tissue, while the errors due to exosmosis from the protoplasm as well as those due to diffusion from the intercellular substance are eliminated."

As disks of tissue vary in thickness, errors arising from this cause were eliminated by first performing an experiment with sea water and then using the same disk for an experiment with one of the salts examined. The rate of change of conductivity during the experiment with the salt solution was then divided by the rate of change in conductivity during the preliminary period when sea water was used. Experiments were also made with dead tissue.

The principal objection that can be raised to this method is that there is no guarantee that the salt which diffuses into the cells on the lower side of the *Laminaria* thallus is the same salt which diffuses out from the cells on the upper side of the thallus. Possible combinations between salt and cell constituents are completely neglected. In short, the complexity of the system is disregarded, and the disk of tissue, several cells in thickness, is regarded as a simple membrane. How far this is justifiable is doubtful.

The method has also been used to examine the permeability of cell walls, bulb scales of *Allium* being employed for this purpose (Brooks, 1917 c).

14. THE DETERMINATION OF PERMEABILITY TO DISSOLVED SUBSTANCES BY MEASUREMENT OF THE ELECTRICAL CONDUCTIVITY OF LIVING TISSUES

A method for the measurement of the electrical conductivity of living tissues has been elaborated by Osterhout (1912 *a*, 1913 *a*, 1914 *j*, 1918 *c*, 1921) who regards the electrical conductivity of a tissue as a measure of the permeability of the protoplasm. Most of Osterhout's measurements have been made with *Laminaria* thallus, but in the latest of the papers cited above apparatus is described suitable for use with other tissue, while a few experiments have been made with animal tissue (1919 *c*). The experimental material most usually employed consisted of a pile of about 80 to 200 circular disks cut from the thallus of *Laminaria Agardhii* and arranged to form a cylinder like a pile of coins, the column of disks being kept in position by means of glass rods. At each end of the cylinder, and separated from it by a small length of solution, was a platinum electrode coated with platinum black contained in an electrode holder of hard rubber. The measurements of electrical conductivity were made by means of Kohlrausch's method.

The question at once arises how far the electrical conductivity of tissue is a measure of the permeability of the protoplasm contained in its cells. This question has been discussed by Stiles and Jørgensen (1918) who have pointed out various difficulties which stand in the way of accepting the electrical conductivity as an exact measure of permeability. The electrical conductivity of a piece of tissue will depend on the conductivity of a number of different phases in the tissue, and we must consider the possible changes in all these phases when the conductivity alters. An increase in conductivity of living tissue is interpreted by Osterhout as an increase in the permeability of the protoplasm to ions. Now an increase in conductivity could be brought about in a number of ways apart from an alteration in the permeability of the protoplasm. Thus a change in the state of aggregation of the contents of the cell wall by which the content or nature of electrolytes was altered, as, for example, by diffusion or by breaking down of complex compounds, would alter the conductivity. This is by no means to be ruled out as a possibility having regard to the complexity of the cell wall (cf. Chapter VII). Experiments with dead tissue (cf. Osterhout, 1918 *a*) in which it is shown that dead tissue behaves differently from living tissue are not convincing evidence' that the changes observed in living tissue are not in any way con-

nected with the cell wall, for in the present state of our knowledge it is not altogether certain that the cell walls of dead tissue are in the same state as those of the tissue before death. Even if the cellulose should be unaltered by the method of killing the tissue, other constituents of the cell wall and its general physical properties may be changed.

But changes in the content and nature of the electrolytes in the protoplasm and cell sap are likely to contribute much more than changes in the cell wall to alterations of the conductivity. Anything, such as diffusion, which brought about an increase in the quantity of free electrolytes in the protoplasm or cell sap, would raise the conductivity, and so would the breaking down of large complex molecules to smaller ones which undergo more ionisation or which give more mobile ions than the complex molecules from which they are derived. Such a change in the protoplasm might well involve an increase in its permeability, but the mere fact that its conductivity was higher, due to its higher content of ions or of more mobile ions, is not in itself to be interpreted as a proof of greater permeability.

How difficult it is to interpret the results obtained by the conductivity method may be exemplified by reference to an experiment made by Osterhout (1918 b) in which *Laminaria* tissue was transferred from artificial sea water containing sodium chloride to a similar solution having the same conductivity but in which lithium chloride was substituted for sodium chloride. This transference resulted in a fall in the resistance of the tissue, while on transferring the tissue back to the original medium the resistance rose.

Osterhout states that these effects are clearly due to diffusion. He says that "the smaller molecules of LiCl diffuse in faster than NaCl can diffuse out, causing a temporary excess of salt, which lowers the resistance." Now whatever the reason for the change in resistance the facts cannot be as Osterhout supposes, for the coefficient of diffusion of lithium chloride (in spite of its "smaller molecules") is slightly lower and not higher than that of sodium chloride as shown for diffusion in water by Öholm (1905) and for diffusion in gels by the present writer. It is nevertheless encouraging that Osterhout acknowledges that factors other than permeability of the protoplasm can affect the electrical conductivity of tissue.

In short, therefore, in the opinion of this writer, while changes in the conductivity of tissues may be correlated with similar changes in the permeability of the tissues, there appear to be so many other possible explanations of changes in electrical conductivity of tissue,

that conclusions relating to permeability changes based on the assumption that the electrical conductivity is a quantitative measure of permeability to ions must be accepted with very great caution.

This concludes our review of the methods that have been devised for the study of absorption of dissolved substances and the permeability of the cell to such substances. These methods are very varied and many of them decidedly ingenious. From this survey it is obvious that reliable data are often readily obtainable with regard to qualitative relations in permeability, and the more important qualitative results have been recorded in the course of this chapter. But when we consider quantitative relations in absorption and permeability the case is very different. Although many results have been recorded professing to give quantitative data with regard to intake of dissolved substances and the permeability of cells to such substances, yet in most cases the presence of disturbing factors renders the results open to criticism. The reason for this is to be found in the complexity of the system involved, combined with the very general assumption that the conditions are simple. Thus, to take only one case, that of the plasmolytic method, it is assumed in this method that the substance which enters the cell passes through a protoplasmic membrane and then accumulates in the cell sap as such, and that there are no disturbing factors to complicate this simple arrangement. Exosmosis, which certainly takes place, is neglected. The possibility of combination of the diffusing substance with a cell constituent or its adsorption with such a cell constituent, which we have seen may certainly take place in certain cases, is also neglected.

In the next chapter, where the quantitative relations of the cell to dissolved substances are considered, it will therefore be necessary to subject the data dealt with to some scrutiny, in order to have as clear an idea of what the results obtained really mean, as the difficulty and complexity of the subject make possible.

(To be continued)

PHYSIOLOGICAL STUDIES IN PLANT ANATOMY

VI. ETIOLATION

By J. H. PRIESTLEY and J. EWING

CONTENTS

INTRODUCTION

THE complex phenomena of plant growth may be regarded as a dynamic equilibrium between the internal tendencies inherited by the plant and the external factors operating during its development. If this conception be applied to the analysis of the Angiosperm seedling given in the first paper of the series(15), in which it was described as an axis capped by two opposing, differently constituted, apical meristems with an equatorial region of food supply, the question immediately arises as to what extent external factors influence the subsequent development of the seedling. It is evident that normally the two apical meristems continue their growth under very different conditions, the stem meristem rising into the light and into comparatively dry air, the root meristem continuing its growth in darkness and in a moist atmosphere. The investigator has to hand experimental methods which will enable him to ascertain whether the characteristic behaviour of these different meristems is inseparably associated with the natural environment; and such experiments have frequently been carried out. The problem requires consideration anew from the standpoint adopted in these papers, and the results of some such experiments will now be considered. Few new observations fall to be recorded, but as the standpoint from which the facts are analysed is all important it seems desirable to make this clear by a discussion of our own experiments before attempting any discussion of earlier work.

The experimental results requiring discussion are those obtained when a stem apex is allowed to develop in darkness, when the charac-

teristic growth phenomena usually included under the phrase " etiola-
tion" effects are obtained. In the case of the root meristem, by the
method described in an earlier paper (Priestley and Tupper-Carey(18)),
its development in light and relatively dry air is readily secured, but
the resultant structural changes are usually not great. In the case of
Vicia Faba L., two seem worthy of note in connection with the sub-
sequent discussion: (1) the primary endodermis appears sometimes
to be slightly later in developing opposite the xylem, as the Cas-
parian strip is frequently formed opposite the phloem before it can
be seen opposite the xylem; (2) small tubercles were frequently noted
close behind the growing apex, but their development never pro-
ceeded far, and the tissue of the tubercles never rose more than one
millimetre above the surface of the root.

THE EXPERIMENTAL STUDY OF ETIOLATION

The discussion in the next section makes it very clear that an
understanding of the causal factors at work in producing the struc-
tural changes observed in etiolated plants has been much hindered
by the very varied nature of etiolation effects in different plants.
The discussion therefore in this section will be limited to the very
characteristic type of reaction under etiolation conditions provided
by a large number of plants of which the broad bean, *Vicia Faba* L.,
or the potato, *Solanum tuberosum* L., may be selected as typical.
Here the comparison will not be with other types of etiolation effects,
but mainly with the normal green plant, and to some extent with
plants not so markedly affected by etiolation conditions such as the
scarlet runner, *Phaseolus Multiflorus* Willd.

In the broad bean and potato the obvious effects of growth of the
stem in continued darkness may be summarised as follows: the stems
become greatly elongated and lose their angular or winged outline,
being round or oval instead: the leaves are very little developed and
remain yellow in colour: the stem apex narrows very suddenly upon
the turgid stem, giving it a pinched appearance: in the broad bean
the plumular hook of the embryo persists during growth. The stems
are extremely rigid and are obviously turgid with sap, bleeding freely
when cut across. Structurally xylem and sclerenchyma are not de-
veloped to such a marked extent as in the green plant, and a hollow
pith is absent or late in development.

These facts looked at from the standpoint of a former paper
(Priestley and Tupper-Carey(18)) suggest a tendency on the part of
the shoot meristem to behave more like a root meristem when

growing under conditions more normal to the root. In the case of the broad bean the apical meristem evidently remains in the same condition in which it was withdrawn from between the cotyledons, the plumular hook and its attendant leaf initials almost completely failing to grow, whilst the tissues of the stems are formed by the activity of the basal cells of the meristem situated below the curved apex. This lack of growth by the upper and more superficial cells of the meristem suggests that the nutrient sap supplied from the cotyledons is percolating among the protoplasts of the meristem only with difficulty, and experiment supports this assumption. If an acid dye such as acid green is driven into the top node of these etiolated shoots under pressure, a pressure greater than one atmosphere, maintained for twenty-four hours, fails to drive the dye into the meristem of the growing points, and the meristem cells above the termination of the vascular strand remain quite unstained. On the other hand, in the course of a few hours a pressure of half an atmosphere drives the dye through the apex of a green shoot grown in the light, when applied under the same conditions, and the meristem cells are soon deeply stained right out to the surface.

The difficulty with which the nutrient sap penetrated the apical meristem of the root was attributed to the different composition of the cell walls intervening between the dense protoplasts of the meristem, and a similar cause is probably operating in the etiolated stem apex. In the root this different composition of the meristem wall was always associated with a characteristic anatomical feature. Immediately behind the growing apex a cylinder of cells differentiates from the meristem into a "primary endodermis" with Casparian strip (Priestley and North (17)). This early differentiation of a functional endodermis undoubtedly plays an important part in the future development of the root, and its own development seems to be causally connected with the nature of the apical meristem, although the chain of causation has not yet been fully traced. In the normal Angiosperm stem on the other hand when growing in the light a functional endodermis with Casparian strip is usually not present, its place being taken by the "starch sheath." If, therefore, the stem apex of the broad bean, when growing in continued darkness, is correctly described as developing more like a root apex, it might be anticipated that the etiolated stem would contain close behind its growing apex a functional endodermis. Perhaps the most interesting point to record in the present paper is that an anatomical investigation of the etiolated stem of the broad bean and of other plants

showing the same type of etiolation has fully confirmed the correctness
of this line of thought. The stem of the etiolated broad bean seedling
possesses a fully developed functional endodermis from its base to
close behind the growing apex. On the other hand, in *Phaseolus
multiflorus*, the apex of the etiolated plant is readily penetrated
throughout by an acid dye when driven in under pressure, the
plumular hook does not persist so long, the leaves open much more
widely, though yellow in colour, and both etiolated hypocotyl and
epicotyl of the epigeal seedling are free from any trace of a primary
endodermis.

As is pointed out later, the presence of such an endodermis in
some etiolated stems has been noted previously, but its significance
has remained unnoted and in most cases its presence has not even
been detected. Hand sections cleared in boiling potash or Eau de
Javelle, and then stained with safranin, gentian violet or other basic
stains, show the endodermis very clearly. Sections stained in
phloroglucin and mounted in strong hydrochloric acid demonstrate
it clearly without clearing, as the contracted protoplasm invariably
stretches tangentially across the cell, remaining firmly attached to
the Casparian strip. In any case of doubt certainty may be obtained
by carefully macerating a fresh section in concentrated sulphuric
acid, when the Casparian strip persists as a characteristic network
(Priestley and North(17)).

On the other hand, in some stems, such as *Phaseolus multiflorus*,
which do not show such striking etiolation phenomena, the apical
development, even in darkness, pursues another course and no
functional endodermis appears in either the etiolated or the normal
stem. Earlier papers in this series (Priestley and North(17) and
Priestley and Woffenden(19)) have demonstrated that the presence
of an endodermis has considerable influence upon the further struc-
tural development of the organs in which it develops. It now remains
to show that the main structural features connected with etiolation
phenomena such as shown by the broad bean may be associated
with the presence of this endodermis.

In the first place this endodermis restricts the nutrient sap neces-
sary for growth to the tissues within the endodermis and to the apex.
We have here a natural cause of the excessive elongation of the stem,
as the only meristem cells receiving adequate supplies of food for
growth are those at the base of the apical meristem which caps the
end of the endodermal cylinder. Growth of this meristem adds to
the length of the stem, and growth in length is therefore favoured at

the expense of the normal lateral growth of leaf and cortex. The collenchyma of the cortex is ill-developed, the angles of the stem opposite the main vascular bundles fail to develop, and the stem remains rounded in outline in cross section.

In *Pisum sativum* L., and in *Vicia Faba*, on continued growth in darkness, the Casparian strip may cease to form although growth still continues, the substances necessary for its formation (Priestley and North(17)) being possibly no longer available. Whenever the endodermis thus fails to form whilst growth still continues, its absence is indicated by the prompt appearance of these angles upon the stem and the greater development of the lateral leaf initials, the buds in their axils also frequently growing out. The same phenomenon may be seen in the potato shoot grown under normal conditions of light. Even under those conditions the first part of the shoot appearing from the tuber must develop in darkness, and so we find at the base of the normal green shoot a small region, usually a few centimetres in height, possessing a primary endodermis, its presence being indicated superficially by the unopened nodes, by the roundness of the stem and by the absence of the prominent stem wings, characteristic of the upper region of the stem possessing a starch sheath.

In view of the considerations advanced in an earlier paper (Priestley and Woffenden(19)) the capacity of the cortex of an etiolated stem to form cork might be expected to be extremely limited. Observation suggests that the fatty substances available for the formation of a cuticle are less in amount, the cuticle being very thin in the etiolated plant; and when this cuticle was removed from the stems of normal and etiolated potato plants, the injured surfaces examined in section three weeks later presented very different appearances. The outer cells were suberised in the normal manner in each case, but whilst in the green stem the phellogen had formed some six to eight layers of periderm by tangential division, in the etiolated plants tangential divisions had only taken place once or at most twice. If, however, cuts were made in the etiolated stems so as to penetrate the endodermis, reparation of the cut by means of a cork phellogen was very active indeed, and in the same period of time more tangential divisions had occurred at the injured surfaces of the etiolated stems than at the surface of the green plants cut in a similar manner. Another interesting result was noticed in these experiments: when the cuts were made through the endodermis of an etiolated stem of the potato, even in the middle of an internode, after the cork had formed over the cut surface branch shoots frequently developed from

the region. This phenomenon seems explicable on the grounds that, in the region of the cut surface, the nutrient sap, restricted normally within the endodermis, is able to irrigate the protoplasts of the cortex. The unusual quantity of solutes suitable for growth then supplied to the cortex not only provides for the activities of the cork phellogen, but leads to the development of the branch shoots.

We are led, indeed, by our experimental results to this conclusion: the sap supply in the vascular strand, with its accompanying organic solutes, is necessary for the vigorous development of the tissues of a vascular plant. When the sap is retained within a functional endodermis only the parenchymatous tissues within that endodermis are capable of active growth. If, however, the sap is able to irrigate the cortical parenchyma, exogenous meristems are able to develop, and these give rise either to lateral shoots or leaves. In the uninjured stem the functional endodermis completely retains this nutrient sap within it, and on the basis of the previous argument this condition should lead to an active formation of endogenous roots from the stem (Priestley and Pearsall(20)). The most superficial observation shows that this is in fact the case. If the etiolated stems are growing in relatively dry air, these endogenous roots appear to be unable to break through the stem surface, but can be detected in the region of the nodes as whitish spots below the cuticle. If the plants are grown in a humid atmosphere these roots readily break through the stem surface. They then develop in great numbers as the stems grow, arising in acropetal succession, not only from the nodes, but frequently in the internodal regions also. An exaggerated development of roots may be obtained by allowing the buds upon a potato tuber to develop in the dark under water. Development will not continue long under such abnormal conditions, but it will continue long enough to give a group of short shoots with two or three internodes, which bristle with lateral roots. It is interesting to note that none of these roots arise directly from the tuber. The development of the tuber is being studied in this laboratory, and its appearance is coincident with a disappearance of the endodermis present in the slender underground tuberiferous stem.

Finally, reference may be made to the axillary branching that is frequently to be seen in etiolated potato plants, and occasionally in etiolated broad beans. Considered in the light of the experiments just described and the observations just recorded, it appears that the branching can hardly be considered as analogous to the normal production and development of axillary buds. In these etiolated plants,

especially in the potato, the endodermal cylinder is very strongly distended by the exudation pressures generated by the sap within. If the stems are cut off and glass tubes attached by rubber connections, relatively large quantities of sap can soon be collected. Under these conditions any weak places in the endodermal cylinder will obviously be severely tested and will possibly break down. It is suggested that the endodermal cells, just above the insertion of the leaf rudiment, represent such weak spots, that frequently the endodermis breaks down in this region under the strong internal hydrostatic pressure, and that the cortical region is then irrigated with nutrient sap, and a lateral shoot develops. In the case of the bean it is suggestive that the lateral branches usually appear at upper nodes where, as has already been pointed out, the endodermal cylinder fails to form.

It is also inevitable that the sap pressure in etiolated shoots should have a marked influence on growth. In comparison with the normal shoot our experiments make one point quite clear: the reduced nature of the transpiring organs of etiolated shoots, and the depression of transpiration incidental on darkness cause a more or less constant sap pressure, so that growth in length or extension goes on equally by day and by night. In the green shoot, however, extension goes on for the most part by night, and is very much reduced or comes to a complete standstill during the day. In the case of *Phaseolus multiflorus*, on which some of our measurements were made, for the first two weeks of growth the nightly increments in extension in the green seedlings were equal to the daily or the nightly increments in the case of the etiolated seedlings. Thus in the early stages the rate of extension in the etiolated seedlings was approximately twice that of the green seedlings. As growth proceeds, however, the development of side axes and leaves on the green seedlings renders comparison of the relative rates of growth practically impossible.

Discussion

The development of ideas as to the causes of etiolation will now be briefly traced; a more complete bibliographical treatment of the subject will be found in the memoir by McDougal (10). It will be convenient to follow the method used by Sachs (22) in the paper which may be regarded as laying the foundation of modern investigation of etiolation, and to consider separately the problem as it affects leaf and stem respectively. Sachs also distinguished between the different types of reaction to continued darkness shown by plants of various

growth habit, and Wiesner[26], when making a comparison between growth in darkness and growth in a saturated atmosphere, distinguished still further types of reaction to continued darkness. This varying behaviour shown by different plants under the same conditions, increases the complexity of the problem, and is perhaps responsible for the fact that some investigators have taken refuge in purely teleological explanations of etiolation, *e.g.* Francis Darwin[4] and Godlewski[6]. From the present point of view teleological arguments are inadmissible, and an explanation in terms of causal anatomy is advanced for one type of growth reaction distinguished by Sachs and Wiesner, viz. that very common type which is exemplified by the broad bean and potato.

The Etiolated Leaf

Sachs distinguished between leaves which emerge from the shelter of the bud at an early date and continue their growth fully exposed to light when distributed along an extended stem. Continued darkness completely prevents the further development of a rudimentary leaf of this type. On the other hand, leaves that grow by the activity of a basal region which remains sheltered at the base of a shortened internode continue to grow in darkness.

The broad bean is representative of the type in which the leaf initial is carried into the light by the elongating internode, and in this type most observers will agree with Sachs' earlier statement that this type of leaf completely fails to develop if maintained in continued darkness. Confusion has arisen in certain cases, and Sachs himself reported later that the leaf of *Cucurbita Pepo*, previously described as undeveloped, reached almost its full size in the dark. Frank[15] (*loc. cit.* p. 395) found the etiolated leaves of this plant of the normal undeveloped type, but Jost[7] and Teodoresco[24] have since reported that the leaves reach their normal size in the dark if provision is made for their nutrition. These conflicting observations appear to be due to neglect to distinguish between the growth of leaves upon a bud which has never been exposed to the light and upon a bud that has been for a short period at any rate in normal daylight before being maintained in continued darkness.

Bataline[1] thought that failure of leaf rudiments to develop under etiolation conditions arose from the inability of the meristematic cells to divide, but Prantl[14] (*loc. cit.* p. 384) has shown by actual countings, that there is a considerable if slow increase in the number of meristematic cells, but that these cells never undergo the increase

in size consequent upon vacuolation. These results point to the
inability of the nutrient sap to reach the leaf meristem, such an in-
ability as is suggested by the observations and experiments described
in the previous section, and it depends in all probability upon the
nature of the wall between the protoplasts in the etiolated meristem.
But if the leaf rudiment is exposed to light even for a short time,
this characteristic of the etiolated wall disappears, the nutrient sap
readily penetrates the meristem and subsequent development of this
leaf is possible even if the leaf is retained in darkness. There is there-
fore no essential contradiction in the different results reported by
the observers quoted above.

The undeveloped leaf rudiments of the broad bean or other plant
of this type when etiolated should therefore consist of dense protein-
filled protoplasts with little water or inorganic salt present because
they are cut off from the sap rising from the roots. Such chemical
data as are available seem to support this conclusion and emphasize
the difference between this leaf type under etiolation and the Mono-
cotyledon leaf with basal growth. Thus in *Vicia Faba* L. the water
content of etiolated leaves is lower than that of young green leaves
(Palladin(11)), while Karsten(8) has shown that etiolated leaves of
Phaseolus, and Godlewski(6) that etiolated leaves of *Raphanus* con-
tain less water than the green leaves. Palladin(12) gives the following
figures for percentage protein content in fresh weight: broad bean,
green leaves 4·95, etiolated 8·38; wheat, green 1·99, etiolated 1·28.
He also finds no soluble carbohydrate in the etiolated leaves of the
broad bean, whilst in wheat he finds an amount equivalent to 2·67
per cent. of the fresh weight. Weber(25) shows that etiolated leaves
contain a lower percentage of ash than normal green leaves, being
especially deficient in calcium.

THE ETIOLATED STEM

Sachs(22) again creates two categories: (1) stems which usually
develop within the sheath of enveloping tissues, and which in con-
tinued darkness extend in length still more; (2) stems normally de-
veloping in the light on which darkness produces little or no effect.
Thus epigeal seedlings are less affected by growth in darkness than
hypogeal seedlings, and seedling shoots are usually much more
affected by growth in darkness than shoots from tubers (*e.g. Dios-
corea*). The broad bean falls in Sachs' first category, and many
attempts have been made to explain the remarkable elongation re-
sulting from growth in darkness. Kraus(9) thought that elongation

might be due to the greater stretching of the less durable cell walls under the pressure of the turgid pith and was naturally puzzled how to explain the lack of extension of the leaf. Rauwenhoff[21] agrees as to the lack of thickening of the cell wall in the etiolated stem, but considers that the cortex contributes to the expansive pressure which stretches the walls and that negative geotropism also plays a part now that it is not inhibited by counteracting heliotropic stimuli. In the light of observations in the first part of this paper it is clear that the excessive elongation in the broad bean type of etiolation must be traced to more definite structural causes and in part to the presence of a functional primary endodermis confining the growth to an extension in length as the result of the activity of an apical meristem, whilst structural developments in the cortical region are cut down to a minimum.

The presence of an endodermis has been noted by Costantin[2] in the case of certain underground stems as in *Rubus* (*loc. cit.* p. 24). In *Ricinus* he finds the endodermis continuous in subterranean stems, interrupted in etiolated stems, and absent in those grown in the light From Costantin's data it seems clear that many underground stems, such as those of *Anemone nemorosa* and *Mercurialis perennis*, have a primary endodermis, whilst the aerial shoots are lacking in this respect. In other cases the buried rhizome possesses a secondary endodermis, *e.g. Galium cruciatum, Achillea Millefolium.* Costantin, however, does not indicate in any way the structural results following from the presence of the endodermis, and indeed seems to suggest (*loc. cit.* p. 47) that in the aerial stem the endodermis has passed on to a further stage, whilst the functional primary stage or later suberised stage persists underground, whereas the important fact is that the endodermis never has been present as a functional layer within the aerial stem. Costantin's observations seem to indicate in many further cases the presence of an endodermis below ground and its absence above. Thus in *Genista sagittalis*, and many other Leguminosæ, he notes the change in outline of the stem in cross section from round in the underground region to angled or winged above, which coincides exactly with our own observations on the broad bean and potato. He also reports the suggestive fact that in many underground stems the cortex is exfoliated as the result of the activity of a deep-seated periderm. The suggestion that this periderm is pericyclic and arises within a functional endodermis is therefore irresistible. In the case of species of *Potentilla* this has been found to be true, and other examples are being examined as opportunity occurs.

McDougal[10] has some very interesting observations which can probably be explained in the same way. In *Apios Apios, Castanea dentata* Marsh, *Hicoria minima* and *H. ovata, Quercus palustre* de Roi, and *Q. rubra* L., he reports a deep-seated periderm in the etiolated plants, whilst the normal stems possess superficial periderms. Unfortunately McDougal did not make any close histological observations so that it is left for subsequent investigators to see whether this deep-seated periderm in the etiolated plant arises within a functional endodermis.

Further evidence of the restriction of growth activity to regions within the endodermal cylinder in this type of etiolated plant is provided by the excessive production of roots already reported. Adventitious root production under etiolation conditions has frequently been noted (Sachs[22], Godlewski[6]), and its relation to the presence of the functional endodermis can hardly be regarded as other than that of effect to cause.

Whilst the standpoint of this discussion assumes that the elongating etiolated plant does not necessarily grow more actively than the plant in the light, but rather redistributes its growth activity in another way, Coupin[3] has recently advanced another explanation of the excessive elongation in etiolation. His hypothesis, supported by some experimental data, assumes that in the green plant growth is less because retarded by some inhibiting substance formed by the chloroplasts in light. Coupin claims that, by adding to the nutrient medium of etiolated seedlings an extract from green leaves of the same species grown in light, he is able to control the growth of etiolated shoots and keep them of normal length. We have attempted to repeat Coupin's work as accurately as his very general description of his experiments has permitted, but our results have been entirely negative. In our experiments with *Pisum sativum*, 70 gms. of green pea shoots and leaves were chopped up and ground in a mortar. To this was added 30 c.c. of tap water and the mixture autoclaved for 20 minutes. It was then put through a press and the thick syrupy liquid extracted was diluted 1 : 9 with tap water and again autoclaved. Eight small flat glass jars were then fitted with covers of sheet cork perforated to allow the radicles of the germinating peas to pass through. Two of these jars were filled with the diluted extract of green peas, two with Shive's optimal solution[23], two with Shive's supra-optimal solution, and two with tap water. The shoots in all cases were very nearly of a uniform length. The roots, however, showed considerable difference in growth. Those grown in tap water

showed most vigorous growth, and then in order, those in Shive's optimal solution, those in Shive's supra-optimal, while those grown in the pea extract showed a very dwarfed and unhealthy condition, as if the extract had some strongly toxic action on them. It was thought that the high osmotic concentration of the extract and the lack of oxygen might have had some inhibitory effect on the growth of the roots, and the experiment was accordingly repeated, the original extract being this time diluted 1:99 with tap water and oxygen bubbled through the liquid. The controls were set up the same as previously. In this case the roots growing in the extract showed slightly more growth, but the shoots attained a length uniform with those of the controls. Until further details of his experiments are given by Coupin we can only conclude that the shortened growth of his etiolated stems was merely an expression of the toxic effect on growth produced by the solution applied to the roots. In any case Coupin's suggestion that the excretion from the chloroplasts in the light thus supplied to the etiolated plant prevented it assuming its normal etiolated appearance would be difficult to reconcile with the frequently observed experimental fact that etiolated portions of plants, organically connected with another part of the same plant growing in the light, continue to show all the normal phenomena of etiolation.

In conclusion it is submitted that the account given above of the causal sequence of events leading to the etiolation phenomena characteristic of plants of the broad bean type, gives a consistent picture of the structural and developmental features brought into play as the result of growth in darkness. In the light of the developmental sequence at the stem apex and the consequent structural alteration behind that apex, all the characteristic phenomena of etiolation of this type may find their explanation. Other growth habits will lead to other types of response to etiolation, and in particular the etiolation habit of the Monocotyledon with its basal leaf growth is reserved for later consideration.

Similarly special cases, such as the lack of response to etiolation in *Humulus* (Sachs(22)), will also be dealt with on another occasion. These allied problems have already been under investigation, and it is hoped to show later that they admit of solution, from the standpoint of causal anatomy, without assumptions that conflict with the general hypothesis presented in this paper, which itself is the natural consequence of the direction from which the general phenomena of plant anatomy have been under investigation in this series of papers.

It is clear without further discussion that the changed behaviour of the developing membranes of the etiolated tissue is very suggestive in relation to etiolation phenomena in widely removed groups of plants.

SUMMARY

1. The plant may be regarded as a dynamic equilibrium between internal inherited factors and the external environment during development.

2. The root and stem normally develop in very different environments: if these are reversed experimentally, an opinion may be formed regarding the extent to which the normal structure is determined by internal factors.

3. If the root is grown in light and air relatively little structural modification can be seen in it.

4. But if the stem is grown in darkness very great structural modifications are produced: these are known as the phenomena of etiolation.

5. Various types of etiolation are exhibited by plants of different growth habit, but in this paper the discussion is restricted to that very common type exhibited by the broad bean and potato.

6. It is shown that in this case an explanation is possible in terms of causal anatomy on the assumption that when the stem apex grows under conditions more normal to the root apex, its development proceeds in a manner more characteristic of the root.

7. Thus the walls between the protoplasts of the apical meristem remain relatively impermeable to the nutrient sap. The "plumular hook" therefore persists and meristematic tissue active in growth is only found below it.

8. The rudiments of the lateral leaves and axillary branches therefore fail to develop further; these etiolated leaf rudiments are rich in protein, but lack water, carbohydrates and inorganic salts.

9. Another consequence of the changed meristematic development is the production of a functional primary endodermis in the stem. To the presence of this endodermis may in part be attributed the reduced cortical development and the lack of angular or winged contours of the etiolated stems in cross section.

10. Adventitious roots develop freely in the etiolated plant as the result of the distribution of the nutrient sap determined by the presence of the endodermis; there is good reason to think that a pericyclic periderm, as opposed to a normal periderm, may often arise as the result of the same factor.

11. Owing to the depression of transpiration extension goes on in the etiolated plant equally by day and night; in the green plant extension goes on mostly by night.

12. Earlier theories advanced to account for this particular group of etiolation phenomena are reviewed from the new standpoint, and some further experiments briefly described, which do not support an alternative hypothesis that elongated growth in etiolation is due to the absence of a growth inhibiting secretion formed by the chloroplasts in the light.

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LABORATORY NOTE

AN APPARATUS FOR THE MEASUREMENT OF STEM ELONGATION

By C. HUNTER AND E. M. RICH

IN the course of an investigation recently carried out in this depart-
ment it became necessary to compare the rates of elongation of
the stem of *Impatiens Balsamina* during relatively short intervals of
time. The horizontal microscope proved to be unsuitable for this
purpose and the various forms of auxanometer were found to be
insufficiently sensitive. Eventually a satisfactory apparatus was
devised which, it is thought, may be of use in laboratory practice.

The lower end of a piece of glass tubing (*C*) 5–7 mm. in length,
drawn out as fine as possible, and sealed off in a flame, rests in the
axil of a small leaf in close proximity to the growing point. *C* is
maintained in a vertical position by means of a glass tube (*T*) of
slightly larger diameter which is attached to the edge of a microscope
slide by means of Canada Balsam. The upper end of *C* bears a square
horizontal platform of mica (*M*) whose side is 0·5 cm. On the upper
surface of *M* a small flat piece of platinum foil (*E*) is fixed with
shellac. A fine insulated copper wire is in contact with the platinum
foil and is connected at its other end to a resistance (*R*). This is con-
nected through a galvanometer (*G*) with a battery of dry cells (*B*),
and to a spherometer (*S*) rigidly clamped above the plant and adjusted
so that its central point is immediately above the platinum electrode
E. The attachment *CME* can slide freely in *T*, and, consequently, as
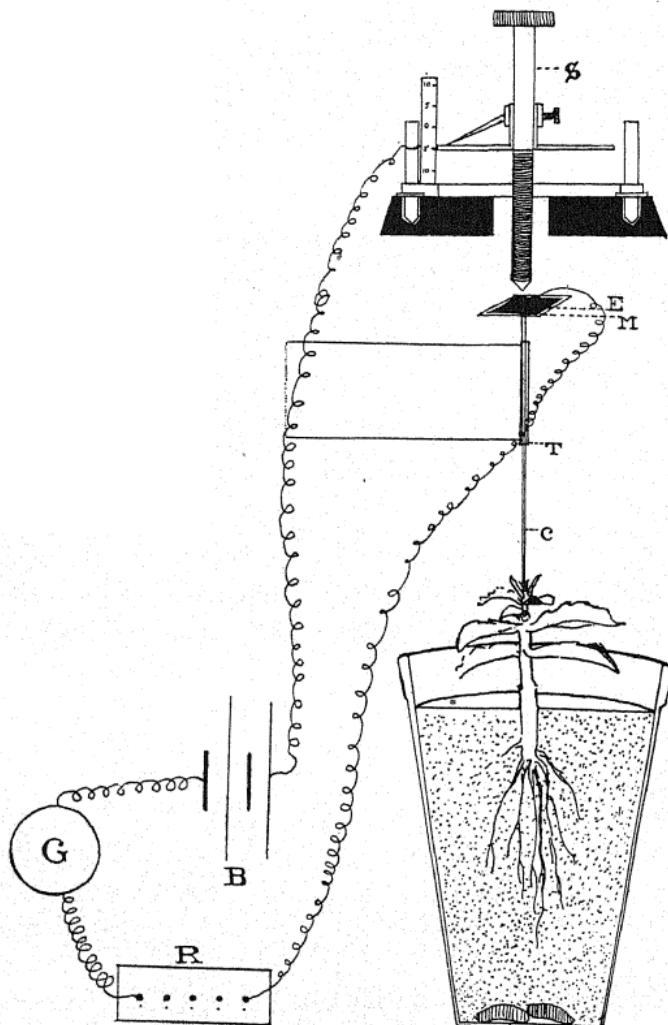
the stem elongates the platinum electrode E is raised the corresponding height. The spherometer is adjusted so that contact is just made between its central point and E—this is indicated by the deflection of the needle of the galvanometer owing to the completion of the circuit. The reading of the spherometer and the time are noted. The circuit is then broken by withdrawing the centre point of the sphero-meter a fraction of a millimetre—this distance is duly recorded,

Stem Elongation
of
Impatiens Balsamina
at 23° C , May 13th, 1922; 11·45 a.m – 12·30 p.m.
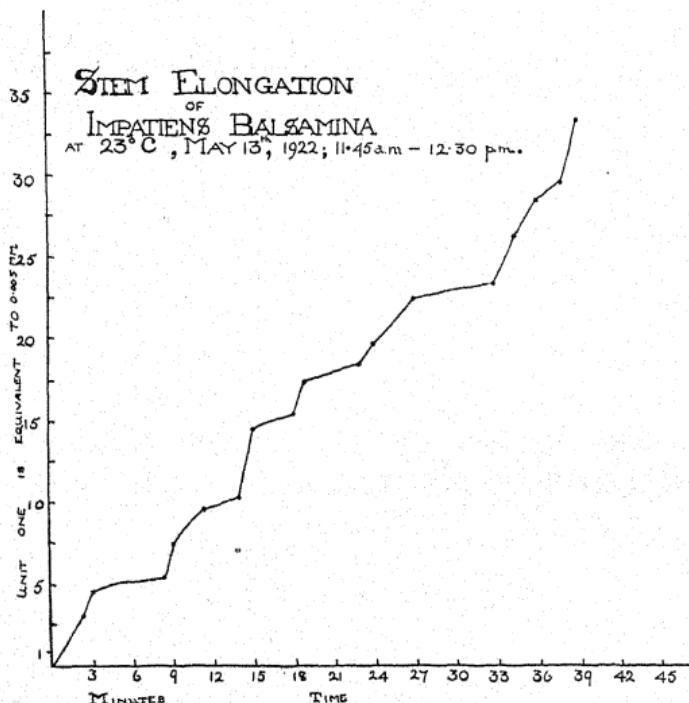
observation kept on the galvanometer and the time noted when the circuit is completed again owing to the raising of the electrode due to the growth of the stem. The operation is then repeated and thus the rate of growth of the stem can be accurately determined.

The spherometer employed was graded into 0·005 mm. units, but even smaller distances than these might be measured by suitable vernier attachments. Corrections due to the interfering effects of fluctuations in temperature on the metal supports and on the glass

tube C during the course of the experiment can be applied. The platform CME must be constructed so as to be as light as possible in order that no undue strain is made on the plant. It was found possible to construct a platform with a weight of 0·035 gm. which caused no injury to the plant.

The order of the results obtainable by means of this apparatus is indicated by the accompanying graph. Obviously the apparatus can only be employed with plants similar to *Impatiens* where the lower end of C can be inserted in the axil of a leaf close to the growing point.

A lamp, or other indicator, to show when the circuit is completed, can be substituted for the galvanometer, but the latter was found to give the best results.

Thanks are due to Mr H. E. George of the Department of Physics, who has assisted in devising this apparatus.

HIATT BAKER LABORATORY, DEPARTMENT OF BOTANY,
UNIVERSITY OF BRISTOL.
December, 1922.

FORTHCOMING MEETING OF THE BRITISH ASSOCIATION, 1923, AT LIVERPOOL

PROVISIONAL ARRANGEMENTS FOR SECTION K

A T the forthcoming meeting of the British Association for the Advancement of Science at Liverpool (September 12–19) the Botanical Section will be presided over by Mr A. G. Tansley, F.R.S. The programme promises to be an interesting one, and it is hoped to make it representative of the chief branches of Botany. In recent years joint discussions between two Sections have been a feature of these gatherings, and at Liverpool it is expected that a discussion on "Virus Diseases" will be arranged with either the Section of Physiology or Agriculture. Much important work of great biological interest has recently been done upon these strange maladies, and a stock-taking of present views upon them is considered to be opportune. Plant physiology will again occupy a prominent place in the programme, and contributions have already been promised by Dr F. F. Blackman and by Prof. V. H. Blackman. The latter will

discuss the relation of plant growth to assimilation, and also the scientific aspects of electro-culture. Certain soil problems on which our knowledge is now rapidly advancing will, it is hoped, be dealt with in papers by Prof. Priestley, Dr Pearsall and Dr Salisbury, who will discuss the relative importance of hydrogen-ion concentration and basic ratios as criteria of plant habitats. A session will be devoted to problems of a morphological nature, and another to mycology.

Section K instituted the "popular" lecture long before it became customary in other Sections, and this year it is hoped that Dr W. L. Balls will speak on the subject of "Cotton." Botanists, as usual, will be well provided with excursions, and the local secretaries are arranging journeys to the famous sand dunes near Liverpool, and to Great Orme's Head on the Welsh coast. In addition there may be a short expedition to the Isle of Man for biologists at the end of the meeting.

An innovation this year will be the exhibit, during a soirée, of laboratory apparatus and specimens, to which botanists are invited to contribute. The usual botanical exhibits will be on view in the spacious rooms of the Hartley Botanical Laboratories, where the Section will meet.

F. T. BROOKS,
Recorder of Section K.

DEPARTMENT OF BOTANY,
UNIVERSITY OF ALLAHABAD.
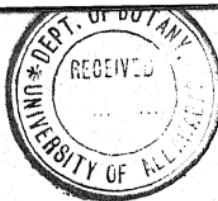
AN EXAMPLE OF LEAF-ENATION IN *ALLIUM URSINUM* L.

By S. L. GHOSE, M.Sc. (PANJAB)

Selwyn College, Cambridge

I N May, 1922 the late Mr Arthur Shrubbs, of the Cambridge
Botany School Museum, found an interesting example of a leaf of
Allium ursinum L., which had developed a second "lamina" on the
lower surface. It was found growing on a boggy patch of ground near
the Whittlesford Railway Station, about eight miles from Cambridge.
Unfortunately, the specimen as brought into the laboratory was not
complete, as the underground portion of it was left behind. In spite
of a careful search by me in the same place a few days afterwards,
the underground portion could not be discovered on account of the
thick undergrowth, nor was any other similar specimen found. It
was suggested to me that the vascular anatomy of the leaf might
be interesting to work out, so I gladly undertook to do it. This paper
embodies the result of the investigation.

According to Mr Shrubbs's observation, the leaf while on the plant
took up nearly an upright position, so that both its surfaces were
turned more or less towards the light. "Petiole" and "lamina" were
alike abnormal in structure. The "petiole," instead of being semi-
circular in section, as in normal examples of *Allium ursinum*, was
flattened and appeared as if made up of two fused "petioles." In a
transverse section at the base of the aerial region it showed three
prominent "ribs," one central and two marginal (Fig. 2). The central
one continued into the "laminar" portion as the "midrib," and
developed on the lower side the second blade. This lower blade was
formed up to the very apex of the leaf, but was only half as broad
as the upper one. The two marginal ones continued into the upper
blade up to the very apex and formed two lateral prominent "ribs"

DFPART'''''T OF BOTANY

—one of the features distinguishing this specimen from a normal leaf of *Allium ursinum*.

I cut transverse sections of the abnormal leaf at different levels to see the behaviour of the vascular bundles. Fig. 2 shows the transverse section at the place where the petiole was broken off. There are three series of bundles. The uppermost consists of two or three weak bundles with phloem towards the periphery; a few phloem elements sometimes occur on the lower side and on the flanks. The middle series consists of a large number of bundles, orientated like those of the uppermost series. The lowermost series is made up of 10 or 12 bundles, which, however, have an orientation opposite to that of the first two series. Higher up the bundles of the uppermost series unite with some bundles of the middle series, so that near the "laminar" portion the petiole has only two series of inversely orientated bundles.

Fig. 3 shows the transverse section of the region where both blades are well developed. Here each blade has one series of bundles, but the orientation is opposite. There are two interesting points to be noticed in this section. Firstly, the stomata are found on the lower surface in the upper blade, but on the upper surface in the lower blade. Secondly, in the upper blade, the xylem of the bundles is found next the lower side, and not next the upper side as is usually the case in leaves. In order to explain these peculiarities, the structure of a normal leaf was studied. The anatomy of the genus has been well described by Irmisch (1850, pp. 1–25), Menz (1910 and 1922) and Arber (1918 and 1920). In *Allium ursinum*, in the underground portion the leaf begins at the base as a closed sheath round the apex of the condensed stem, and the bundles are arranged in a ring with their phloem towards the periphery. Higher up the leaf becomes thicker on one (dorsal) side and thinner on the opposite side. In the thicker side another series of bundles is developed towards the periphery by the splitting up of some of the bundles of the main series (Arber, 1920, Fig. 28 *c*). The orientation, however, is similar in both series. As we go higher up the ventral side gets gradually thinner till finally it disappears. The thicker side becomes rounded off and forms the "petiole" of the leaf (Arber, 1920, Fig. 28 *d*). In the "petiole" the bundles of the second series unite with some of those of the main series, so that near the limb only one series of bundles is left (Arber, 1920, Fig. 28 *e*). In the limb there is no differentiation of the mesophyll into palisade and spongy parenchyma, and there is only one series of bundles to be seen, but the phloem is

mostly situated next the upper side,
while the xylem is towards the sto-
matal side (Menz, 1922, Text-fig. 2 *b*).
This condition has been brought
about by torsion in the "petiole"
and has been noted before by Goebel
(1905, p. 296). Here the morpho-
logically upper side has developed
the structure of the morphologic-
ally lower side. Keeping this fact
in view it becomes clear that in the
case of the abnormal leaf the ap-
parently upper surface of the leaf is
morphologically the lower surface.
Thus the smaller blade has in reality
been produced on the morphologic-
ally upper surface of the leaf, but,
most probably on account of torsion
of the petiole below the point where
it was broken off, it appears to be
on the lower surface of the leaf.

Not only has this abnormal leaf
developed a second blade, but it
has also formed a second "petiole"
to support it (Fig. 1). This splitting-
up of a "petiolar" region of a leaf
seems to be unique. A large num-
ber of examples of leaf-enations
have been described, both from the
upper and from the lower surface,
in monocotyledons as well as in
dicotyledons, but in no case is
there any record of the enation ex-
tending below the insertion of the
"lamina." Masters (1869), Worsdell
(1915), Celakovsky (1884 and 1892),
Velenosvky (1907, pp. 408–11) and
Buchenau (1888 and 1891) have
described and figured a number of
leaf-enations, and I have examined
all the examples of leaf-enations in
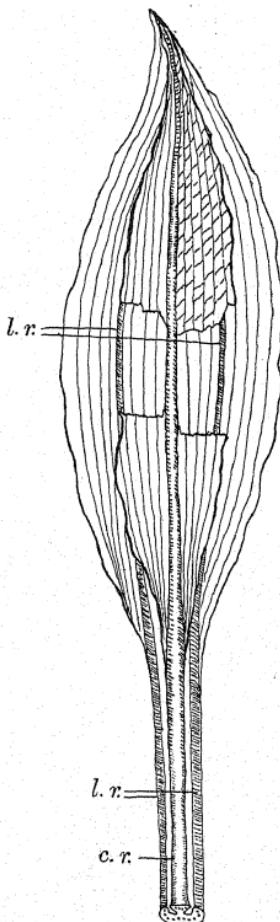
Fig. 1. *Allium ursinum.* The abnor-
mal leaf as seen from the lower sur-
face. Portions of the lower blade are
cut off to show the lateral "ribs" of
the upper blade. *c.r.,* central "rib";
l.r., lateral "ribs." × ¾.

4—2

the British Museum (Natural History), London, but neither is a leaf
of *Allium ursinum* included amongst these, nor is there any case in
which the enation is continued below the base of the "lamina."
The fact that this example of a leaf-enation, which is continuous
in both "petiole" and "lamina," is found in a monocotyledon is
interesting in connection with the Phyllode Theory of the mono-
cotyledonous leaf, as will be seen later on. It may be mentioned
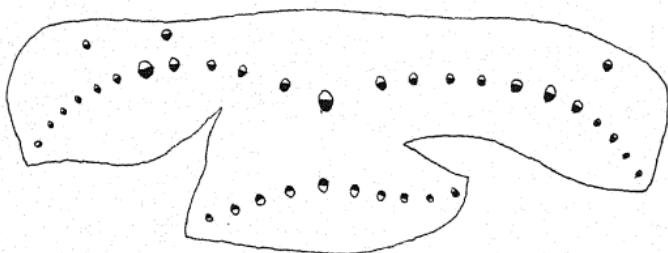
Fig 2. *Allium ursinum.* Transverse section of the double "petiole" at the
region where it was broken off. × 15.
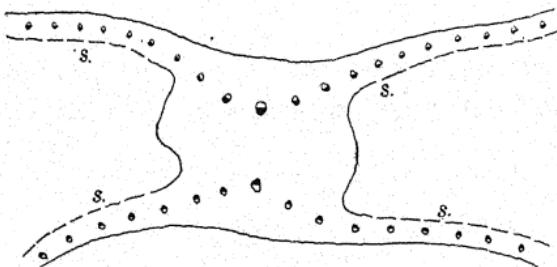
Fig. 3. *Allium ursinum.* Transverse section of the "laminar" portion of the
double leaf. *ss.*, stomata. × 10.

here, however, that Worsdell (1915, **1**, p. 201) records the case of
a double leaf in a vine, in which there was a double petiole, but the
two blades were quite free from each other. As, however, it was un-
known what position on the stem this leaf occupied, it is doubtful
whether it was an example of a leaf-enation or a fusion of two leaves
by their petioles only. Celakovsky (1884, Figs. 38, 39; and 1892,
Fig. 17) also figures some leaves which had developed a small second
blade from the petiolar region. But these enations were absolutely

free from the main blade, and were produced at a much lower level than the latter. His figure (1884, Fig. 70) of a double leaf of *Tulipa*
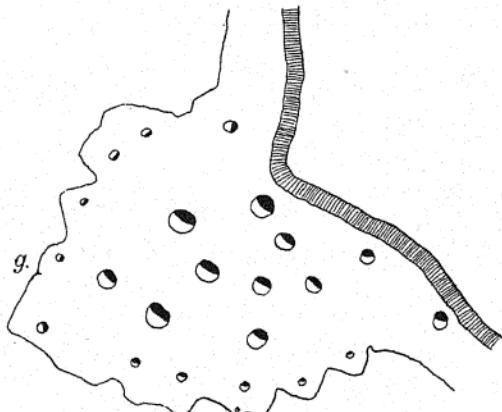
Fig. 4. *Xanthosoma appendiculatum.* Transverse section of the double-bladed leaf just below the origin of the second blade. Region of the palisade parenchyma is shown by vertical lines. ×20.
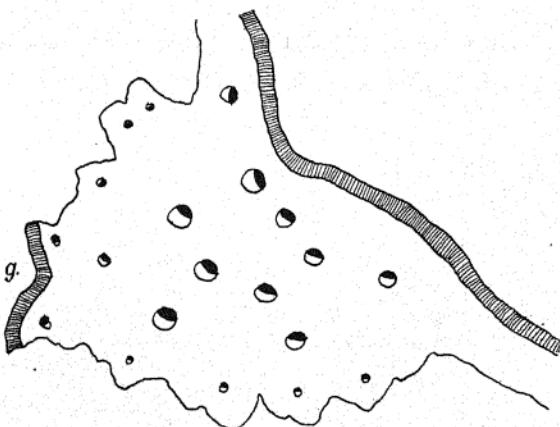
Fig. 5. Transverse section of the same a little higher up. ×20.

sylvestris seems to suggest a double and flattened condition of the "petiole," but unfortunately the diagram is not complete.

Theoretical

There are one or two points which seem to be of some theoretical interest in the anatomy of this abnormal leaf. In this example of enation the law of laminar inversion, according to which opposed laminar surfaces are similarly constituted, is obeyed in spite of the fact that the torsion of the "petiole" brings about some complication in the general arrangement of tissues. This law has been seen to hold good in nearly all the cases of enations mentioned by Worsdell (1915) and others. For comparison, sections were cut of a double-bladed leaf of *Xanthosoma appendiculatum* Schott, and I found that it obtained there also. Another interesting thing attracted my attention in some of the sections of the latter. Fig. 5 shows a transverse section of the leaf at the region of the base of the second blade. The "midrib" shows a number of invaginations all round, which seem to suggest a tendency to form more blades. The "petiole" shows no such invaginations. I cut a section of a normal leaf at a similar region and noted that it also showed these invaginations, though in a less marked degree. The genus *Xanthosoma* has already attracted attention on account of its tendency to produce leaf-enations. Worsdell (1915, 2, p. 168) refers to the remarkable enations from the lower surface of its leaf. Velenovsky (1907, pp. 410–11) mentions the double-bladed leaf in *X. atrovirens*. Willis (1919, p. 692) mentions the pocket-forming habit in *X. appendiculatum*. Whether or not more than two blades are ever formed in *X. appendiculatum* I cannot say, but these invaginations seem to suggest that here a process of grooving, associated with development of wings, has been adopted for the formation of the "lamina," as has been found to occur in the Palms and in some Irids by Arber (1921 and 1922). A series of sections through the abnormal leaf of *X. appendiculatum* shows this quite clearly. Just below the base of its second blade the "midrib" shows a number of ridges and invaginations all round the lower surface (Fig. 4). As we go higher up, one of these grooves situated near the middle of the lower surface becomes more marked, the epidermal cells round it become larger, the hypodermal cells slowly change into palisade parenchyma (Fig. 5), and the two ridges are finally transformed into wings, so that a second blade is produced. Thus it is clear that this second blade is produced as a result of grooving or invagination, associated with development of wings. A little higher up, this groove closes, and the "midrib" has again only one blade, indicating that the second blade is only an elaboration of the two ridges pro-

duced by the invagination. I may note here that Willis (1919, p. 692) attributes this phenomenon of the formation of the second blade to the "tangential division of the embryonic leaf." Obviously, I cannot accept this explanation. In view of the above explanation of the formation of the second blade, it is possible to conceive that the normal blade also is the result of the expansion of the two ridges, which are
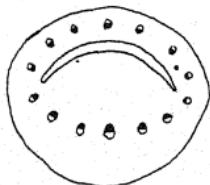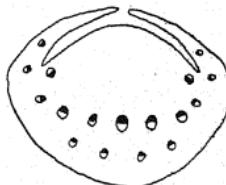
Fig. 6   Fig. 7
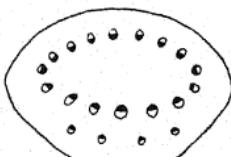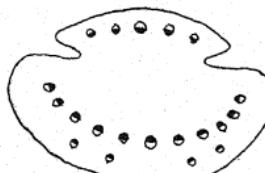
Fig. 8   Fig. 9
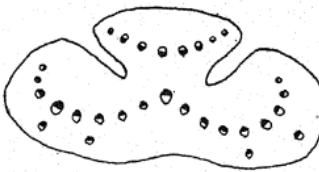
Fig. 10

Figs. 6–10. Hypothetical figures to show the various stages in the doubling of the "petiole" by the agency of two opposite invaginations.

produced by a median groove on the upper surface of the apical part of the "petiole."

Now returning to the abnormal leaf of *Allium ursinum*, an examination of Fig. 2 suggests that a process of invagination has been at work in it also. Although, unfortunately, I could not secure the lower portion of the "petiole," yet it seems to me that this apparently double nature of the "petiole" is due to the production of two grooves

on opposite sides. It may be suggested that this abnormality has been brought about by fusion of two opposite leaves by their "petioles" and "midribs"; but it does not seem likely, as there is no indication whatsoever in the internal tissues to show that any fusion has taken place, while the three-"ribbed" condition of the "petiole" can easily be brought about by a process of flattening, which is accompanied by the production of two opposite invaginations. Figs. 6–10, which are merely hypothetical, might very well represent what has actually happened in the specimen. Figs. 9 and 10 are fairly comparable with some diagrams given by Arber (1921, Figs. 56, 57) in connection with the formation of blades by this process of invagination amongst the Irids. Celakovsky's diagrams of sections of the leaves of *Crocus* and *Ferraria* (1892, Figs. 32 and 40) are also interesting in this connection. They suggest the possibility of the formation of two blades by such a process of invagination. The bundles, however, seem to have similar orientation, most probably on account of the non-completion of the vascular ring in the "petiole," and marked growth of tissue at the "open" region. The orientation of bundles may be different according to the plane of the invagination, the completion or otherwise of the ring of vascular bundles in the "petiole," and the growth of various tissues at the time of expansion. In the case under discussion the last named factor may have been influenced by the "law of laminar inversion." It may not be out of place to mention here that in the normal leaf of *Allium ursinum* the ring of bundles in the "petiole" is not complete, hence the leaf does not show "phyllode structure," as has been noted by Arber (1920). Menz (1910, Text-figs. I–IX) gives some figures of *Allium odorum* L., in which this ring is nearly complete, so it seems not very improbable that in the case under discussion the ring may have been completed abnormally. Menz (1922, Text-fig. 3) also gives a diagram of a transverse section of a leaf of *Allium nigrum* L., a member of the same section of *Allium*—Molium—as *Allium ursinum*, which shows two series of bundles with opposed orientation, again showing the possibility of the completion of the ring of bundles in the abnormal "petiole" of the latter. It is possible to imagine that if the pair of invaginations had not existed in the abnormal case of *Allium ursinum* and the apex of the "petiole" had just expanded into one limb, the limb might have shown "phyllode structure," as has been noted in many species of *Allium* by Arber (1920) and Menz (1922).

In a recent paper, Gaisberg (1922) questions the validity of the Phyllode Theory and gives many arguments in support of the Midrib

Theory. The two blades of the abnormal leaf, however, are more easily explained as expansions of the apical part of the "petiole" than as those of the "midrib," because the petiole itself is flattened, and has three prominent "ribs," the lateral ones of which are continued only in one of the blades. Moreover, the continuity of double nature in "petiolar" and "laminar" regions would seem to suggest that the "petiole" and "lamina" are morphologically the same.

SUMMARY

1. In this paper an example of leaf-enation in *Allium ursinum* is recorded for the first time.

2. The "doubling" is found not only in the "laminar", but also in the "petiolar" region. This seems to be unique, because no example of leaf-enation is found to be on record, which is continuous in both "petiolar" and "laminar" regions.

3. There is one series of bundles in each of the blades, but their orientation is opposite: thus the sport obeys the "law of laminar inversion, according to which opposed laminar surfaces are similarly constituted." This law has been found to hold good by Worsdell and others in nearly all the examples of leaf-enations.

4. The "doubling" is explained as the result of formation of two opposite grooves in the "petiole," and the development of wings in its apical portion by the four ridges thus formed.

5. An example of leaf-enation is described from *Xanthosoma appendiculatum*, which is also explained as the result of grooving, associated with development of wings. It is also suggested that it is possible to conceive that the normal blade has also been produced in the same way. This is interesting in view of Arber's description of similar methods being employed by Palms and some Irids in the formation of "pseudo-laminæ."

6. This only recorded example of the enation extending down to the flattened "petiolar" region, being found in a monocotyledonous leaf, indirectly lends support to the view that the "lamina" in monocotyledons may be only a modified portion of the petiole.

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THE TAXONOMY AND VARIATION OF THE
GENUS *MICROCYSTIS* IN CEYLON

By W. B. CROW

A RECENT examination of the collections of freshwater algæ from the plankton of the inland waters of Ceylon, made by Prof. F. E. Fritsch in 1903, showed that the members of the genus *Microcystis* form probably the most important constituents of the phytoplankton during the period (Aug. 21st to Nov. 10th) at which the collections were made[1]. The species evidently attained a remarkable abundance in many localities frequently forming water-bloom. Along with this abundance in number of individuals was found an extraordinary diversity of form, the various types being linked together, however, by intermediates. In fact, whenever abundant material occurs the precise limits of the different species become impossible to define. This is particularly true of those species which possess pseudovacuoles, since these species are found in the greatest abundance. The same phenomenon has been observed for three species from tropical Africa and has led Ostenfeld[6] to consider all the forms with pseudovacuoles as belonging to the same species. Transitional forms connecting not only these three species but also several other types of a very varied nature will be recorded below. This kind of continuous variation is being discovered to a greater and greater extent in *Cyanophyceæ* and *Chlorophyceæ*. That it is not fluctuation directly dependent on environment is shown by the fact that forms which are quite distinct, together with transitional types, may exist for considerable periods, side by side, under the same ecological conditions. It is probable, therefore, that there is some genetic difference between the types distinguished as species below.

In order to emphasise the more essential specific distinctions among the members of the genus the following key is given. Although any such scheme must be largely artificial its primary divisions are based on cell characters as being more fundamental than colony characters, which have been too greatly relied upon by previous investigators. The key includes several species which are not known from Ceylon but also the two new species described below. As the

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[1] The material was preserved in tubes of dilute formalin and that dealt with here was in an excellent state of preservation.

above remarks will have made clear the species of *Microcystis* can
only be distinguished definitely when a considerable amount of
material is available and individual variation is taken into account.
Moreover, there has been considerable difference of opinion with
regard to the limitations of most of the species, and some attempt
has been made to enumerate the essential features of the species met
with in the Ceylon collections.

KEY TO THE GENUS *MICROCYSTIS*.

A. Cells spherical.
  a. Cells containing pseudovacuoles.
    α. Cells 3–7µ diam.
      1. Colonial envelope definite, refractive, often stratified.
        i. Colony generally compound .   .   .   . . *M. viridis*
        ii. Colony generally simple .   .   .   . *M. marginata*
      2. Colonial envelope less definite, colonies compact.
        i. Colony not greatly lobed nor very elongated.
          * Colony clearly clathrate .   .   .   . *M. æruginosa*
          ** Colony not clearly clathrate .   .   . *M. flos-aquæ*
        ii. Colony elongated to many times its diameter, segmented into
          partial colonies .   .   .   .   . *M. pseudofilamentosa*
        iii. Colony very greatly lobed, in the form of cuneiform figures.
          * Colony clearly clathrate .   .   .   . *M. ochracea*
          ** Colony not clearly clathrate .   .   . *M. scripta*
      3. Colonial envelope quite indefinite, cells very loosely scattered
                               M. protocystis
    β. Cells less than 3µ diam.
      1. Colonies generally compound .   .   .   . *M. ichthyoblabe*
      2. Colonies simple, pellicular .   .   .   .   . *M. firma*
  b. Cells not containing pseudovacuoles.
    a. Cells distinctly yellow   .   .   .   . *M. fuscolutea*
    β. Cells not yellow, mostly pale blue-green.
      1. Cells 5–5·5µ diam.   .   .   .   . *M. pallida*
      2. Cells less than 3µ diam.
        i. Colony elongated to many times its diameter . *M. stagnalis*
        ii. Colony not greatly elongated.
          * Colony free-floating, clearly clathrate . *M. holsatica*
          ** Colony free-floating, or attached to stones, not clathrate
                             M. pulverea
          *** Colony attached to water-plants .   . *M. parasitica*
B. Cells elongated .   .   .   . *M. elabens*

    The above includes all the fully known freshwater species. For
other forms consult Tilden[8] and Forti[2].

MICROCYSTIS ÆRUGINOSA KUETZ(4).

Including var. *major* Wittr. (see (2)), Fig. *a*

Colonies roughly spherical or ellipsoidal, generally clathrate. Cells 3–7 μ diam., spherical, with pseudovacuoles. This must be regarded as the typical form. The well-known figure of Kirchner(3) does not agree with that of Kuetzing(4). It was frequent in all the following localities although never reaching the abundance of *M. flos-aquæ*.

Loc. Tank[1] Andangawa-mahawewa on small jungle footpath from Habarane to Sigiri, Oct. 11th; tank Balaluwewa, Sept. 29th; margin of tank Andankulam, about three miles from Trincomalie, Oct. 18th; small pond in Botanical Gardens, Anuradhapoora, Nov. 3rd; Rock pool connected with Kalawewa-tank, Sept. 29th.

MICROCYSTIS FLOS-AQUÆ (WITTR.) KIRCHNER(3)

Figs. *b* and *c*

Colonies roughly spherical, ellipsoidal or often squarish in optical section; not clathrate; cells 3–7 μ diam.; spherical with pseudo-vacuoles.

According to Lemmermann(5) the distinction between this species and *M. æruginosa* is in its non-clathrate character. Wesenburg-Lund(9) on the other hand says that both *M. æruginosa* (= *Clathro-cystis* Hansgirg) and *M. flos-aquæ* (= *Anacystis* Hansgirg) often possess clathrate colonies, but that in the latter species this is "generally not very noticeable because these colonies have not the sharp outlines which the *Clathrocystis* colonies have with their distinctly definite covering of slime." Wesenburg-Lund, from a study of a large amount of material from the Danish freshwater plankton, considers that the essential difference between the two species lies in (*a*) the distinctness of the outline of the colonial mucilage, and (*b*) the form of the colonies. He states that the two supposed species are connected by numerous transitional stages, and cites an instance where a colony had one half with the characters of *M. flos-aquæ* and the other half those of *M. æruginosa*. In view of the variability of these two species he thinks that the numerous species described by Lemmermann cannot be upheld. Ostenfeld(6) also argues the identity of all the pseudovacuole-containing species of *Microcystis*, having found transitional forms between *M. æruginosa*, *M. flos-aquæ* and *M. viridis*.

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[1] The "tanks" in Ceylon are reservoirs of partially artificial origin. In them, however, the microorganisms live under normal conditions.

Observations on the Ceylon material show similar transitions. Moreover, it is clear that reproduction of the colonies largely takes place by vegetative breaking. Hence a clathrate colony may break up into non-clathrate masses, which in their turn may undergo the process of reticulation. The forms recorded here as *M. æruginosa* are such as show little or no reticulation.

Most of our colonies had an indistinct outline. But those from Colombo Lake often had a very definite marginal region when seen in optical section. This was especially marked in those colonies that were breaking up into numerous daughter colonies as shown in Fig. *b*. Sometimes the partial colonies were mere nests of less than a dozen cells, surrounded by a wide clear sheath. Formation of daughter colonies often appears localised so that the former may appear as buds upon the latter. Possibly these appearances represent successive stages in the breaking up of the colony. It is only the fact that such small colonies are found in actual process of formation that proves these forms to be connected with typical colonies of *M. flos-aquæ*, for the latter are much larger and have a far more indefinite colonial mucilage sheath.

Other variant colonies of *M. flos-aquæ* approach *M. scripta* and *M. ochracea* in a tendency to produce outgrowths. But the typical lobing of the definite type figured for these latter species was not met with in the collections examined. We have preferred to retain *M. flos-aquæ* as a distinct species since the forms as described above often occur in large quantity and cannot all be explained as developmental phases of other species of *Microcystis*.

Loc. Lake at Candy, Sept. 24th; lake at Colombo, — ; four small tanks near entrance to Botanical Gardens, Anuradhapoora, Oct. 2nd; tank Mineri, Oct. 12th (water-bloom); tank Tissawewa, near Anuradhapoora, Oct. 3rd; small rock pool, in wet season of the year certainly connected with tank Punchi-kekirawa close by, Sept. 28th.

MICROCYSTIS PROTOCYSTIS n. sp.[1]

Fig. *d*

Colonies irregular, often diffuse, with colony-sheath not clearly delimited, sometimes disappearing. Cells very numerous, varying in mode of aggregation from closely crowded to generally dissociated, spherical, 3·5–6·5 μ diam., with pseudovacuoles.

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[1] A Latin diagnosis of this and other new species is given as an appendix.

This species differs from *M. flos-aquæ* and *M. æruginosa* in its more irregular colonies and especially in the predominantly dissociated state of the cells. As some transitional forms of this species closely approach *M. flos-aquæ* it was at first regarded as a form of the latter species. Thus in colonies from Perithpan-pokuna much of the material was comparatively well provided with mucilage, the distances between adjacent cells being about 2–4 times the cell-diameter. In tank Mineri and a pool near tank Punchi-kekirawa typical specimens occurred in association with colonies of the form of typical *M. flos-aquæ*. As, however, very large numbers of loose colonies in the dissociated state shown in Fig. *d* have been observed in material which shows evidence of normal conditions both of growth and preservation, there can be no doubt that *M. protocystis* forms a distinct type. The difference between this and any previously known species is as great as any specific distinction within the genus. In Wesenburg-Lund's (9) description of *M. flos-aquæ* and its forms, he mentions nothing approaching the dissociated condition of *M. protocystis*. The latter must on our present knowledge be considered as a perfectly independent species. The presence of transitional forms resembling those of *M. flos-aquæ* suggests that further investigation may show such forms to be a regular phase in the life-history of *M. protocystis*, but should this prove to be the case it would not be sufficient to show the latter to be identical with typical *M. flos-aquæ*.

M. *protocystis* naturally extends the range of variation known in the genus *Microcystis*. In fact it falls within the generic definition of *Aphanocapsa* as given by some authors. But to place it in the latter genus would be to obscure its natural affinities. The great distance apart of the cells and the quite indefinite colonies are of course features wholly in accord with *Aphanocapsa*. It must be noted, however, that these two characters by no means exclude it from the genus *Microcystis*. The present writer has shown in a previous publication (1) that the distance apart of the cells is one of the least important specific characters in the species of *Microcystis*. It is well known, too, that the definite character of the colony is by no means equally maintained in the species (see *e.g.* figures given here). It might, of course, be supposed that these characters (*i.e.* wide dispersal of the cells, indefinite nature of the colony) were so well developed in *M. protocystis* that it might be regarded as having passed beyond the limits of the genus *Microcystis*, not because there is any great gap between this species and the related members of *Microcystis* but because in it the characters referred to seem to reach their highest

development. The genus *Aphanocapsa*, however, consists of a very heterogeneous assortment of forms some of which will doubtless fall into other genera when their life-history becomes fully known. The genetic connection of some supposed species of *Aphanocapsa* with other Chroococcaceæ (*e.g. Chroococcus, Glæocapsa*) is already established in some cases(5). The presence of pseudovacuoles, the cell size and the resemblance to *M. flos-aquæ* in certain phases all point towards the fact that this new species is a true member of the genus *Microcystis*.

Loc. Tank Mineri, Oct. 12th (water-bloom); tank Andankulam, Oct. 18th (water-bloom); rock pool in wet season of the year certainly connected with tank Punchi-kekirawa close by, Sept. 28th; Perithpan-pokuna near Isurumunija-temple at Anuradhapoora, Oct. 3rd.

MICROCYSTIS PSEUDOFILAMENTOSA n. sp.

Figs. *e* and *f*

Colonies very long and narrow, consisting of a series of partial colonies, *i.e.* constricted at intervals, sometimes considerably widened at places and broken through or reticulate. Margin of colonial mucilage indistinct. Cells $3-7\,\mu$ diam., spherical, with pseudovacuoles. Colonies varying greatly in size, sometimes twenty times as long as wide, frequently $200-300\,\mu$ in length; $20-30\,\mu$ in width, the partial colonies being about equal in length and width.

The colonies of the above species resemble those of *M. stagnalis* Lemm. in their elongated form but not in their segmented character. *M. pseudofilamentosa* is also distinguished from *M. stagnalis* by the size of its cells, which are more than twice as large as in the latter species, and in the presence of pseudovacuoles which do not occur in *M. stagnalis*. From *M. æruginosa* our species is distinguished by the form of its colonies and by the more indistinct margin of the colonial mucilage. *M. pseudofilamentosa*, apart from the latter feature, would resemble, in form, certain string-like bodies described by Wesenburg-Lund for *M. æruginosa*(9). But the mode of origin appears to be different in our specimens. Instead of arising as lateral outgrowths from the rounded colonies of the ordinary *Clathrocystis* as described by Wesenburg-Lund for the forms referred to, our colonies would appear to break up frequently into their partial colonies which divide again to give elongated compound colonies much in the same way as a cell may divide up to form a filament. This character suggested the specific name given. *M. pseudofilamentosa*, in virtue

of its clathrate colonies, may at times closely resemble *M. æruginosa* and *M. ochracea* (Brand) De Toni, but in dealing with these forms it is necessary to take into account the general or normal type of the colony and certainly on this basis *M. pseudofilamentosa* must be regarded as quite distinct. This species and *M. stagnalis* are remarkable examples of homoplasy with the elongated Tetrasporaceæ.

Loc. Tank Nuwaradwewa at Anuradhapoora, Oct. 2nd.

MICROCYSTIS MARGINATA (MENEGH.) KUETZ.(4)

Figs. *g* and *h*

Colonies rounded or irregular, generally flattened and of more or less lenticular form, simple or in clusters not surrounded by a common colonial sheath. Margin of colonial mucilage very distinct. Mucilage very refractive, sometimes stratified. Cells irregularly scattered.

The single colonies were generally ellipsoidal or ovoidal in outline, averaging about 140–150 μ in length, 60–95 μ in width. The cells were generally comparatively large (6 μ diam.) and filled with gas vacuoles. The stratification was often very faint, often absent in the young colonies. The latter were generally found in groups and were often very small. In the younger state the distinction from *M. viridis* is largely one of degree. *M. marginata* is not so abundant as *M. flosaquæ*.

Loc. Perithpan-pokuna near Isurumunija-temple at Anuradhapoora, Oct. 3rd; rock pool connected with Kalawewa-tank, Sept. 29th (rare); small shallow bay of Nuwarawewa near Anuradhapoora, Oct. 2nd (rare).

MICROCYSTIS VIRIDIS (A. BR.) LEMM. (see (5))

Colonies round or rectangular in outline, consisting of a group of partial colonies surrounded by a common sheath. Cells 3–7 μ diam., spherical, with pseudovacuoles. In both this and the previous species the mucilage is highly refractive, clearly defined at the margin and the cells are extremely variable in their grouping (see (1)).

Loc. Small shallow bay of Nuwarawewa near Anuradhapoora, Oct. 2nd.

MICROCYSTIS HOLSATICA LEMM. (see (5))

Colonies spherical or ellipsoidal, clathrate, margin of colonial mucilage clearly defined. Cells about 1 μ diam., spherical or subspherical, without pseudovacuoles. The lacunæ were irregular in form and variable in size, often about ten times the diameter of the cell.

The closely crowded cells are characteristic and distinguish this species from those of *Aphanocapsa* with cells of the same size and somewhat similar colonies.

    Loc. Tank Andankulam, four miles from Trincomalie, Oct. 20th.

MICROCYSTIS PULVEREA (Wood) Migula (see (5))

    Colonies spherical or ellipsoidal, often lobed. Cells $2-3\,\mu$ diam., subspherical, without pseudovacuoles.

    Loc. Lake at Colombo,   —   ; tank at Dambulla, Sept. 9th.

    Var. *INCERTA* (Lemm.) nob. = *M. incerta* Lemm.

    G. M. Smith (7) points out that *M. incerta* Lemm. differs mainly from *M. pulverea* (Wood) Migula in the size of its cells and that it might be better to consider *M. incerta* as a variety of *M. pulverea*. Lemmermann (5) says that the colonies of *M. incerta* are always isolated, those of *M. pulverea* "oft zu vielen dicht nebeneinander liegend." The latter condition was not frequent in our specimens. It therefore seems that the essential difference between the two forms is one of size and as it is not customary to establish species on this character alone we have regarded *M. incerta* as a variety of *M. pulverea*.

    As in *M. flos-aquæ* the smaller colonies, apparently produced by the breaking up of the larger, have much more clearly defined margins than the larger, *i.e.* older colonies.

    Loc. Rock pool connected with Kalawewa-tank, Sept. 29th.

    Forma *ELONGATA* n.f. As var. *incerta* but colonies more greatly elongated, *i.e.* to about three or four times their length, thus approaching *M. stagnalis*, although of course still very much less elongated than the latter.

    Loc. With var. *incerta*.

RELATIONSHIP OF *CŒLOSPHÆRIUM DUBIUM* Grun. (see (5)) TO *MICROCYSTIS*

    This species, which also occurs in the freshwater plankton of Ceylon, shows relationship with certain members of the genus *Microcystis* in the following features: (a) the presence of pseudovacuoles in the cell, (b) the not infrequent grouping of the colonies in clusters, and (c) the size of the cells which averaged $6\,\mu$ as in *M. flos-aquæ*. As the latter species was associated with *C. dubium* in the lake at Candy and tank Tissawewa and as this association has been noted by Lemmermann, a genetic connection would at first sight seem probable. But the older colonies of *C. dubium* always show very clearly a single layer of cells and in these, as in some of the younger

forms, which are not so obviously distinguishable from *M. flos-aquæ*, the colonial mucilage does neither stain with Safranin nor Methyl Violet 5 B, whereas in the latter species the mucilage is irregular and stains deeply with these colouring reagents. The cells in both species stain deeply with Methyl Violet, this stain showing them to be surrounded by an individual membrane of moderate thickness. The presence of an individual membrane around the cells has been used by Lemmermann to characterise the genus *Cælosphæriopsis*. In the latter these cell-envelopes are apparently visible without staining. The presence of individual membranes in *Cælosphærium dubium*, although less obvious, shows there is no fundamental distinction between the two genera. In *Microcystis* we meet with all stages of differentiation of the cell-envelope even in one and the same colony (see figure of *M. viridis* in (1)) and in the Chroococcaceæ in general the presence or absence of such an envelope has been shown to be of little direct systematic importance(1).

C. *dubium* was found in the following localities: tank at Dambulla, Sept. 9th; tank at Haberane, Oct. 10th; Lake at Candy, Sept. 24th; tank Tissawewa near Anuradhapoora, Oct. 3rd.

SUMMARY AND CONCLUSIONS

1. The genus *Microcystis* is represented in the freshwater plankton of Ceylon by at least eight species, two of which are described as new.

2. The species are connected by numerous transitional forms, the nature of which is described.

3. The distinctions between the species of *Microcystis* are of the same kind as between other cogeneric species of *Cyanophyceæ*.

4. The present distinction between *Aphanocapsa* and *Microcystis*, in view of such a species of *M. protocystis* n. sp. is seen to be an artificial one. The former genus consists of forms of various affinities.

5. *Cælosphærium dubium* Grun. is related to the species of *Microcystis* possessing pseudovacuoles, although generically distinct from them. *Cælosphæriopsis* Lemm. is not distinguished by a valid generic character.

In conclusion my warmest thanks are due to Prof. F. E. Fritsch for providing the material for the above investigation.

DIAGNOSES OF NEW SPECIES

Microcystis protocystis n. sp.

Strato libere natante, irregulario, saepe diffuso, tegumento strati non plane delimito, interdum vel nil vel evanescente. Cellulis numero-

sissimis, varie aggregatis, modo densissimis plerumque sparsis, sphæricis, 3·5–6·5 μ diam. pseudovacuolibus provectis.

Loc. In aquis dulcibus, Insula Ceylonica.

Microcystis pseudofilamentosa n. sp.

Strato libere natante, longissimo, angusto, in locis constricto ut series stratorum secondariorum fiat, interdum passim dilato et vel perforato vel reticulato tegumento strati subdistincto. Cellulis sphæricis pseudovacuolibus provectis 3–7 μ diam. Strato magnitudine variabilissimo, interdum vicies sæpe decies longo quam lato, sæpe 200–300 μ longo, 20–30 μ lato; stratis secondariis sæpe 20–30 μ longis.

Loc. In aquis dulcibus, Insula Ceylonica.

Microcystis pulverea (Wood) Migula.

Var. *incerta* (Lemm.) nob.

Cellulis 1–2 μ diam.: cetera ut in typo.

Forma *elongata* n. f.

Strato vel ter vel quater longo quam lato; cetera ut in var. *incerta* nob.

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EXPLANATION OF PLATE I

a. *Microcystis æruginosa* Kuetz. Typical Colony from Tank Balauwewa.
b. *M. flos-aquæ* (Wittr.) Kirchner. Colony from Colombo Lake with Partial Colonies.
c. *M. flos-aquæ* (Wittr.) Kirchner. Typical Colony from Colombo Lake.
d. *M. protocystis* n. sp. Typical aggregation from Tank Mineri.
e. *M. pseudofilamentosa* n. sp. Typical Colony from Tank Nuwarawewa.
f. Another colony of the same, apparently showing method of division.
g and *h.* *M. marginata* (Menegh.) Kuetz. *g.* Side view of typical colony; *h.* Front view of same.

(The scale measures 100μ and is divided into units of 10μ each.)
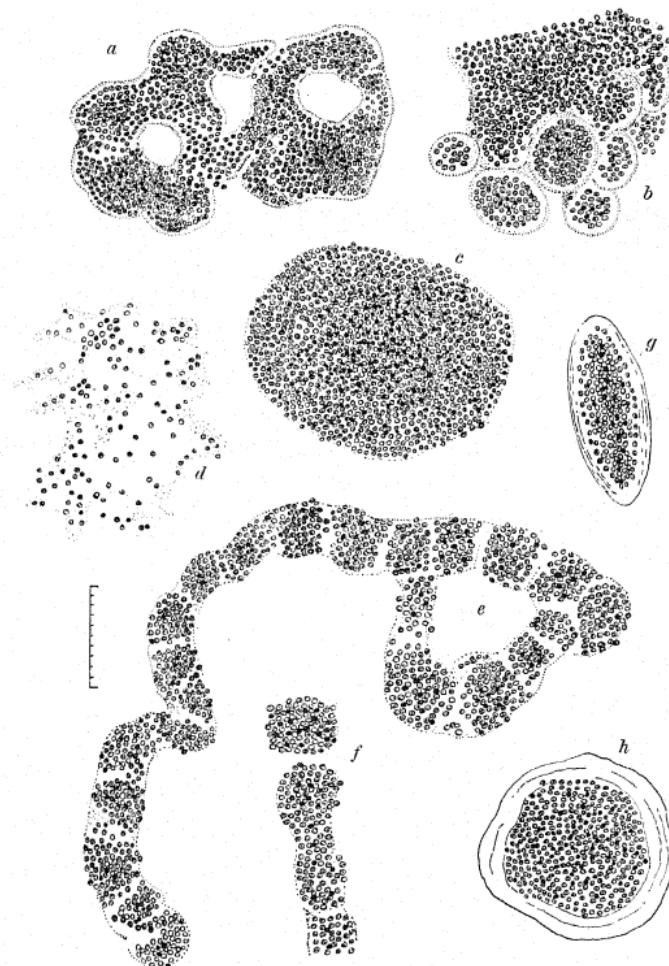
TETRAEDROIDES SPETSBERGENSIS GEN. ET SP. NOV., A NEW ALGA FROM SPITZBERGEN

(RESULTS OF THE OXFORD UNIVERSITY EXPEDITION TO SPITZBERGEN, No. 28)

By B. MILLARD GRIFFITHS, M.Sc., F.L.S.

I N the material collected by Mr Summerhayes during the Oxford University Expedition to Spitzbergen in the summer of 1921, an alga occurred which is apparently a new genus. It was found among the stems of a moss in a crevice of rock on Bear Island, and was associated with a few small diatoms and much vegetable detritus from the plants among whose stems it grew.

At first glance the alga resembles a species of *Tetraedron*, but closer examination shows that the plant is not unicellular but consists of at least two cells and in some cases even three or four. The thallus of the commoner bicellular type is pyriform, bipyramidal or ellipsoidal with slightly truncated ends (Figs. 1–7). The exterior walls of the cells are thick but the interior transverse wall is thin. Other two-celled forms are tetrahedral or pyramidal or irregularly polyhedral with very thick exterior walls (Figs. 8–11). As a rule, one or more angles of the polyhedron are distinctly flattened at the apex. More rarely polyhedral thalli consisting of three or four cells occur (Figs. 12, 13); in these cases also, the exterior walls are thick and the interior walls are thin.

Vegetative reproduction takes place by a kind of incipient thread formation. One or both cells of the thallus grow out into thin-walled tubular extensions (Figs. 14, 15, 16). The tube divides primarily by a series of constrictions, and each segment is again divided into two cells by the formation of a thin transverse wall. The primary constriction may be complete, in which case the end of the resultant thallus is sharply rounded off, or after constriction has partly taken place, the final division is accomplished by the laying down of a thick transverse wall which splits and sets the pairs of cells free. In this case the resultant thalli have the truncated apices to which reference has been made.

In Fig. 17 is shown an apparently abortive attempt at the formation of an incipient thread of about seven cells, of which the three lower have failed to mature. The upper part of the thread shows the

primary constriction of the tube and the thin transverse wall dividing the lower constriction-segment into two cells.

In Fig. 18 is seen a slightly different type of division in which the constriction is partial and the division has been completed by the formation of a transverse wall of considerable thickness. The segment of the initial tube thus cut off is about to be divided by a thin transverse wall whose rudiment can be seen on the left-hand side. The resultant bicellular thallus will have at least one end truncated when the thick transverse wall splits and sets it free.

In Fig. 19 is seen a group of three pairs of cells which have been formed by a process of complete constriction into segments, followed by the division of each segment into two cells by the formation of thin transverse walls.

One case was observed of what may be a type of asexual reproduction (Fig. 20). One of the cells of the common pyriform or ellipsoidal type appears to be discharging four reproductive bodies through a small lateral pore, but the bodies are not of regular form, nor do they appear to be motile. Although careful search was made, no other example was seen.

Each cell of the thallus contains one rather large nucleus. It is provided with a conspicuous nucleolus, and generally lies close to the thin transverse wall. The chloroplasts are small and numerous, more or less oval in shape, and they frequently contain a small pyrenoid (Fig. 7 shows nuclei and chloroplasts; nuclei are also shown in other figures).

The systematic position of the alga is very difficult to determine without a further knowledge of its life-history. It is apparently a reduced member of a filamentous group but beyond that little more can be said with certainty. The only alga showing the least resemblance is one brought to my attention by Prof. Fritch, namely *Peniococcus Nyanzæ* Woloszynska, an alga from the plankton of the Victoria Nyanza (see Woloszynska in *Hedwigia*, Band 55, Heft. 4–5, p. 205, 1914; *Zellpflanzen Ostafrikas*, 1910; *Studien u. d. Phytopl. des Viktoriasees*, Taf. 7, Fig. 14). It is a thin-walled unicellular alga described as "cylindrices, irregulariter emarginatis, medio constrictis; pyrenoide singulo. Membrana hyalina. Chlorophoris multis. Propagatio divisione transversa." In the dividing stage it bears some resemblance to *Tetraedroides* forms as seen in Figs. 1, 2, 3 and 7, but there is no trace of incipient thread formation and the bicellular state is a phase of division and not a permanent condition of the thallus.

The division of *Tetraedroides* by complete or partial constriction may be a modification of the septation method seen in the Siphono-

clades, and the many chloroplasts and pyrenoids and the extrusion of spores from a lateral pore also point somewhat to that group. If so, *Tetraedroides* might be considered as a much reduced member of the Siphonoclades, possibly derived from a *Rhizoclonium*-like ancestor, and bearing a relationship to the group similar to that of *Pleurococcus* to the Ulotrichales. These surmises are, however, far too speculative to use as a definite basis of classification.
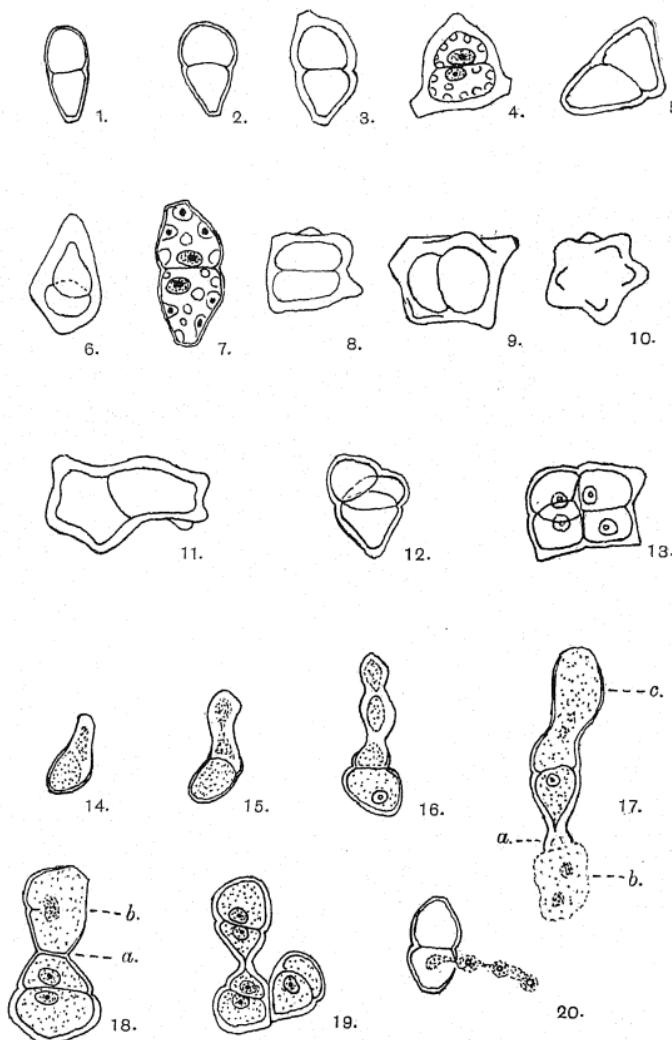
It might be suggested that this alga, whatever its systematic position may be, is a filamentous form which has assumed the bi-cellular thick-walled habit as a protection against the rigour of the climatic conditions prevailing in the situation in which it lives. The incipient thread formation and the relatively thin-walled thalli might be considered as summer stages, and the thick-walled polyhedral forms perhaps as winter stages.

DIAGNOSIS.

TETRAEDROIDES SPETSBERGENSIS gen. et sp. nov.

Thallus piriformis ellipsoidalis tetrahedralis aut polyhedralis, ex cellulis duabus (sed rarius tribus vel quatuor) compositus. Muri exteriores crassi, interiores tenues. Cellula uninucleata; chloro-plastides multæ quarum aliquæ unam pyrenoidem habent. Multi-plicatio vegetationalis per filum incipiens quod in geminas cellulas constrictione et muris transversalibus divisum est. Multiplicatio asexualis per sporus quatuor et nonmotiles qui ex cellula per foramen laterale exeunt. Thallus long. 25–42 μ; lat. 10–25 μ; crass. max. muri 4 μ. Habitatio inter muscos in rimis saxorum in Insula Spetsbergensi.

DEPARTMENT OF BOTANY,
ARMSTRONG COLLEGE, UNIVERSITY OF DURHAM.
January, 1923.

EXPLANATION OF PLATE II
Camera-lucida drawings, × 650

Figs. 1–7. More common type of thallus. In Fig. 4, the nuclei and chloroplasts are shown; in Fig. 7, ditto, some of the chloroplasts contain pyrenoids.

Figs. 8–11. Less common polyhedral thalli; Fig. 10 shows axial view.

Fig. 12. Three-celled thallus; empty specimen.

Fig. 13. Four-celled thallus; nuclei shown.

Figs. 14–19. Incipient thread formation. In Figs. 14, 15 and 16 the cell-contents of the tubular portions are somewhat plasmolysed. In Fig. 17 *c*, the upper part of the thread is segmenting by constriction but the trans-verse walls have not yet appeared. In the lower part, the two lowest cells, *b*, are apparently aborting, and cell *a* is completely aborted.

Fig. 18. Partial constriction and formation of thick transverse splitting wall at *a*. At *b*, thin interior transverse wall is about to form.

Fig. 19. Group of pairs of cells formed by complete constrictions alternating with thin transverse-wall formation.

Fig. 20. Formation of four spores (?), of which three are already extruded from the cell *via* a lateral pore.

PERMEABILITY

By WALTER STILES

CHAPTER XII

QUANTITATIVE RELATIONS IN THE PENETRATION OF DISSOLVED SUBSTANCES INTO PLANT CELLS

WHILE qualitative tests of the penetration of dissolved substances into plant cells have yielded results of considerable interest, we can hardly expect to be able to formulate the laws governing the passage of substances into and out from the cells without adequate quantitative data. These data we most certainly do not yet possess; nevertheless what information we have is of great interest and is sufficient to show that the simple osmotic view of the plant cell is a very inadequate hypothesis and is incapable of affording a complete explanation of the cell in regard to its relations to dissolved substances. Without further preface various aspects of the quantitative relations of plant cells will be dealt with in the following sections of this chapter.

THE UNEQUAL ABSORPTION OF THE IONS OF A SALT BY PLANT TISSUE

In earlier work on the absorption of salts by plant tissue it was assumed that salts were absorbed as such by plants. Of late years, however, it has come to be recognised that there may be an unequal absorption by plant tissue of the two ions of a single salt. In the light of recent knowledge certain observations of long standing become easily explicable on this ground, such as the acidity or alkalinity developed in some water culture solutions in which plants have been growing for a time.

If unequal absorption of ions takes place there are necessary consequences of such a phenomenon. The penetration of an excess of one ion into the tissue cannot, on account of the attraction of the oppositely charged ions, take place without the replacement of this excess by an equal quantity of another ion carrying the same charge.

This replacement can take place in two ways. In the first way an equivalent quantity of hydrogen-ion or hydroxyl-ion, as the case

may be, can appear in the external solution, this hydrogen-ion or hydroxyl-ion being derived from the solvent, in which case the excess of the absorbed ion is accompanied into the tissue by an equivalent quantity of hydroxyl-ion or hydrogen-ion according as the excess of absorbed ion carried a positive or negative charge. In the second way an equivalent quantity of some other ion carrying the same charge as the ion absorbed in excess, diffuses out from the tissue.

To take a definite example, let us suppose that a particular tissue immersed in a solution of potassium nitrate absorbs the nitrate ion in excess. Either an equivalent quantity of hydroxyl ions appears in the solution to balance the excess of potassium ions left there, the excess of nitrate being accompanied into the tissue by an equivalent quantity of hydrogen-ions; or an equivalent quantity of some anion or anions, as, for example, sulphate, malate, citrate, diffuses out from the tissue. If it can be shown that an unequal absorption of ions takes place a complete view of the process can only be obtained by determining the quantities of hydrogen, hydroxyl or other ions that appear in the external solution.

Unequal absorption of ions by isolated tissues. Direct observations on the intake of the different ions of a salt by isolated tissues are few. Some have, however, been made by Meurer (1909) on the absorption of a number of salts by slices of beetroot and carrot 3 mm. in thickness. A few observations have also been made by Ruhland (1909 *b*) on beetroot in the form of slices 3 mm. and 1 mm. in thickness. Some of the results of these authors are summarised in the following table. The absorption in every case is given as the proportion of the concentration of ion absorbed to the concentration of the ion in the external solution.

TABLE XXX
Absorption of Ions of a Number of Salts by Plant Tissue

Tissue	Salt	Concentration of solution	Duration of absorption in days	Relative Absorption		Observer
				Kation	Anion	
Carrot	KCl	$N/15$	2	0·374	0·287	Meurer
,,	NaCl	$N/12$	2	0·411	0·258	,,
,,	$CaCl_2$	$N/14$	2	0·270	0·229	,,
,,	KNO_3	$N/20$	4	0·524	0·570	,,
Beetroot	$CaCl_2$	0·4 %	2	0·258	0·0354	Ruhland

These results, as far as they go, give clear indications of an unequal absorption of ions by the storage tissues examined. The

observations are, however, not numerous, and under the circumstances it would be unwise to draw too far reaching generalisations from them. Indeed, no suggestion of a general rule is apparent from these results. Thus, with carrot the kations of most of the chlorides examined were absorbed to a somewhat greater extent than the chlorion in each case, while with beetroot this difference is very much emphasised in the case of calcium chloride, about seven times as much calcium ion being absorbed as chlorion in the same time. On the other hand carrot absorbed more chlorion than kation from a solution of magnesium chloride (cf. Table XXXI), and slightly more nitrate than potassium from a solution of potassium nitrate.

That this unequal absorption of ions is a property of living cells and not of dead tissue is shown by Meurer's results with living and dead carrot tissue immersed in solutions of magnesium chloride (Table XXXI). Whereas with living tissue about 32 per cent. more anion was absorbed than kation during four days, with dead tissue equivalent quantities of the two ions were absorbed.

TABLE XXXI

Absorption of the Ions of Magnesium Chloride by Dead and Living Carrot

Concentration of solution	State of tissue	Duration of absorption in days	Relative absorption by Kation	Relative absorption by Anion
$N/24$	Living	2	0·327	0·336
	,,	4	0·286	0·377
$N/22$	Dead	2	0·958	0·950
	,,	4	0·953	0·953
$N/95$	Living	2	0·563	0·774
	,,	4	0·577	0·895
$N/105$	Dead	2	—	0·950
	,,	4	—	0·869

Unequal absorption of ions by whole plants. The unequal absorption of ions by *Cucurbita Pepo* was investigated by Pantanelli and Sella (1909). The plants were grown as in water-culture, 16 to 21 individuals being placed in each vessel. After the roots had been surrounded by conductivity water for two days, the plants were transferred to the experimental solutions for a definite number of days, after which time the dry weights of roots and shoots were determined and the external solution analysed for both ions of the experimental salts. The results obtained are summarised in Table XXXII.

TABLE XXXII

Absorption of Ions by the Roots of Living Plants of *Cucurbita Pepo*.

(Data from Pantanelli and Sella)

Salt	Concentration in gm. mols. per litre	Duration of experiment in days	Absorption in mg. ions Kation	Anion
Potassium chloride	0·03	6	23·38	30·68
Calcium chloride	0·02	14	0·00	51·39
Potassium sulphate	0·0188	6	11·6	18·07
Calcium sulphate	0·0165	19	0·00	1·98
Potassium acid phosphate	0·02	10	1·15	49·04
Calcium phosphate	0·032	12	1·10	78·93

These results exhibit very clearly the very great differences which may exist between the quantities of kation and anion absorbed by the roots of living plants in the same time. In all these experiments the anion was absorbed in excess of the kation, sometimes very greatly in excess. Calcium was absorbed either to a very slight extent, or not at all.

This work was later extended by Pantanelli (1915 *a, b, c*) to a number of other species, including freshwater plants (*Elodea canadensis, Azolla caroliniana*), higher land plants (*Allium Cepa, Phaseolus multiflorus, Cicer arietinum, Vicia Faba, Lupinus albus*), yeast of barbera and marine algæ (*Gigartina acicularis, Cryptonemia Lomation, Phyllophora nervosa, Dictyota dichotoma, Ulva lactuca, Valonia utricularis*). The experiments were carried out at temperatures between 15° and 20° C. A wider range of salts was also employed. Although some cases were observed in which equivalent quantities of the two ions of a salt were absorbed, in the vast majority of cases the absorption of the kation and anion was unequal. A number of Pantanelli's results are collected in Table XXXIII.

Pantanelli's results, of which those shown here are only a small selection, show that unequal absorption of the ions of a salt is an almost universal phenomenon, at any rate in the concentrations used. Slight unequal absorption of the constituent ions of calcium chloride by beet, carrot and maize after the roots had been immersed in the solution (initially about 0·04 *N*) for periods varying from 8 to 39 days, was also observed by Johnson (1915). Hoagland (1918) found that barley absorbed more nitrate than sodium from solutions of sodium nitrate, but that the two ions of potassium chloride were absorbed by this plant in equivalent proportions.

TABLE XXXIII

Absorption of Ions by Living Plants. (Data from Pantanelli)

Species	Salt	Concentration in gm. mols. per litre	Duration of experiment in hours	Absorption in mg. ions Kation	Anion
Elodea	Calcium chloride	0·05	2	3·0	0·41
canadensis	Potassium sulphate	0·05	2	3·44	0·29
,,	Ammonium sulphate	0·05	2	0·29	2·05
Azolla	Calcium chloride	0·05	2	0·26	0·00
caroliniana	Potassium nitrate	0·05	2	1·89	0·91
,,	Aluminium nitrate	0·0125	2	0·16	0·23
,,	Potassium sulphate	0·025	2	2·67	2·5
,,	Ammonium sulphate	0·025	2	0·17	2·28
,,	Magnesium sulphate	0·025	2	3·58	2·48
,,	Ferrous sulphate	0·025	2	0·76	0·61
,,	Aluminium sulphate	0·0125	2	0·95	0·062
Phaseolus	Calcium chloride	0·01	8	1·18	1·50
multiflorus	Barium chloride	0·01	8	0·04	0·77
Cicer	Potassium chloride	0·025	8	0·35	0·23
arietinum	Calcium chloride	0·025	8	0·025	0·14
,,	Potassium nitrate	0·025	8	2·74	1·95
,,	Aluminium nitrate	0·0125	8	0·68	2·31
Ulva lactuca	Calcium chloride	0·025*	2	3·29	2·79
,,	Potassium sulphate	0·025*	2	1·48	0·18

* 10 c.c. of 0·25 M salt + 90 c.c. sea water.

The unequal absorption of the ions of calcium chloride by *Pisum sativum* and *Zea Mays* was shown by Miss Redfern (1922 a), who examined the influence of the concentration of the salt on the degree of inequality of absorption by the former species. She found that the more dilute the solution the less the divergence between the absorption of the two ions. Her results for the edible pea are shown in tabular form below (Table XXXIV).

TABLE XXXIV

Influence of Concentration on the Absorption of the Ions of Calcium Chloride by the Roots of Living Plants of *Pisum sativum*. (Data from Redfern)

Initial concentration of solution	Percentage absorption after 36 hours Calcium	Chloride
0·1 N	17·74 ± 1·376	3·578 ± 0·506
0·01 N	19·61 ± 2·33	12·47 ± 1·66
0·001 N	23·10 ± 5·30	15·09 ± 3·736

It appears likely that the excess absorption of one ion is accompanied in some cases by the solution developing an equivalent quantity of hydrogen or hydroxyl ions so that the solution becomes

acid or alkaline, while in other cases the equality of positively and negatively charged ions in the external solution is maintained by exosmosis of ions from the plant cells. The acidity and alkalinity that may be developed in water culture solutions, and which has been noticed from the time of Knop onwards, can be explained as a case of the former happening. On the other hand, Hoagland (1918) found that the culture solution in which barley was growing maintained a neutral reaction, and, if the reaction was acid to start with, the solution became neutral after contact with the roots for a time.

Pantanelli considered that the unequal absorption of the two ions resulted in the development of acidity or alkalinity at first, but that after a time this might disappear and the excess of absorbed ion become balanced by excretion of oppositely charged ions from the tissue.

Miss Redfern found that the calcium chloride solutions used in her experiments in which the calcium ion was absorbed in excess remained approximately neutral throughout the experiment, while tests of the external solution showed that magnesium and potassium ions had diffused out of the tissue. Therefore the excess of calcium ion absorbed is replaced in this case by the diffusion out of the tissue of ions carrying a similar charge, and not by hydrogen ions from the water of the solution.

Stoklasa, Šebor, Týmich and Cwacha (1922) also conclude that the absorption of aluminium and ferric ions by the roots of living plants of *Eriophorum vaginatum*, *Phragmites communis* and *Carex riparia* is accompanied by the excretion of calcium, magnesium and sodium ions. Their conclusion is based on water culture experiments in which the analysis of the solutions was made after the experimental plants had been growing in them for 13 days. Since the aluminium ion is absorbed much more rapidly than the anion, this excretion of other kations must necessarily take place if the solution does not become highly acid or alkaline.

THE POSITION OF THE EQUILIBRIUM ATTAINED IN THE INTAKE OF DISSOLVED SUBSTANCES BY PLANT CELLS

It is a remarkable fact, and one indicating how little the complexity of the problems involved in the phenomena of permeability and absorption have been realised by workers in these fields, that scarcely any of those who have attempted to obtain quantitative data with regard to permeability have concerned themselves with the equilibrium attained in the passage of dissolved substances into

plant cells. Yet it is obvious that a determination of the rate of
intake of a substance can give no indication of the permeability of
the cell unless the position of the equilibrium in the intake is known.
Some few writers have, indeed, had a clear conception of this.
Thus Pfeffer pointed out that a cell might be permeable to a
dye and yet the intake of the dye might be unobservable because
no accumulation of the dye took place in the interior of the cell.
Yet in employing the favourite method used in recent work for
determining the rate of intake of dissolved substances, that is, the
plasmolytic method, investigators most usually do not consider the
position of equilibrium at all, while the possibility of an equilibrium
with different concentrations of the substance inside and outside the
cell, is never considered. Quite usually the rate of intake is regarded
as a measure of permeability. This would only be so if the difference
in concentration between the external solution and the cell sap
remained constant, which, according to the theory on which the
method is based, is not the case. Lepeschkin, it is true, takes account
of the change in concentration difference between external solution
and cell sap as deplasmolysis proceeds, and so in treating his ex-
perimental data, is at any rate consistent with the theory on which
he works.

But is it correct to assume that the dissolved substance diffuses
into the cell until there is equality of concentration of this substance
inside and outside the cell? Pfeffer's work with dyes showed most
undeniably that this is not the case with the majority of dyes
examined by him, while it is equally certain that many substances
occur normally in the plant in considerably higher concentration
than they do in the external medium from which they were obtained.
Wherever chemical combination or adsorption takes place in the
interior of the cell it is obvious that the assumption made in the
plasmolytic and some other methods, that the penetration of the
dissolved substance into the cell can be regarded simply as the
passage through a membrane into a medium which does not react
with the substance, is unsound. It is therefore very necessary that
the evidence available with regard to the position of this equilibrium
should be examined.

We may notice in the first place that a number of observations
are on record which indicate that the concentration of a substance
inside a cell can remain greater or less than its concentration in the
external medium. With animal cells the case of blood corpuscles
and serum may be cited, and with plant cells the observations of

Wodehouse (1917) on the concentration of various substances in the cell sap of *Valonia* (see Chapter XI). The explanation generally offered of this phenomenon is that the cell membranes are impermeable to the substance in question, so that it can exist in different concentrations on the two sides of the membrane. This cannot be the explanation of Pfeffer's results with dyes, where the accumulation of the dye in the cell sap is accounted for by chemical combination of the dye with a cell constituent to form a non-diosmosing compound. But some of those who have employed the plasmolytic and other methods have curiously enough neglected the possibility of such a complication in the intake of the substances they have examined.

We must particularly notice in this connexion the opinion of Moore and Roaf (1908) and Moore, Roaf and Webster (1912), who rejected the theory of a membrane impermeable to crystalloids as the explanation of the permanent difference in concentration on the two sides of the membrane, and who held that the observed results could be adequately, and more satisfactorily, explained as due to adsorption of the penetrating substance by cell constituents or to chemical combination between the penetrating substance and some cell constituent or constituents in the same way as Pfeffer explained the intake of dyes.

The absorption ratio. Actual determinations of the position of equilibrium attained in the penetration of dissolved substances are not numerous, and in practically no case is the method of determining it free from every objection. Such as they are, however, they do provide almost overwhelming evidence to the effect that in no observed case is the position of equilibrium in the intake of any substance necessarily one of equality of concentration in the external solution and the cell sap.

Nathansohn (1903) made chemical analyses of the external solution and the expressed sap of the marine alga *Codium* after immersion for some days in solutions of sodium nitrate. Further experiments were carried out by the same author (1904 *a, b*) on the same lines with slices of tubers of *Dahlia* and *Helianthus tuberosus* and of the root of beet, a number of salts being used. Nathansohn's observations were later extended by Meurer (1909).

The relation between the final internal and external concentrations can best be expressed by the ratio of the first quantity to the second. To this ratio, that of final internal to final external concentration, the name *absorption ratio* has been given by Stiles and Kidd (1919 *a*), who calculated the absorption ratios given by

Nathansohn's and Meurer's experimental results. In Table XXXV are shown a selection of these absorption ratios.

TABLE XXXV.

Absorption Ratios

Tissue	Salt	External concentration	Duration of absorption in days	Absorption ratio	
				Kation	Anion
Codium	NaNO₃	0·5 %	4	—	0·56
,,	,,	,,	10	—	0·68
,,	,,	1·0 %	5	—	0·44
Dahlia	,,	,,	4	0·59	—
,,	,,	,,	6	0·51	—
,,	NH₄NO₃	1·5 %	4	0·32	—
Carrot	KCl	N/15	4	0·548	0·386
,,	NaCl	N/12	4	0·489	0·307

It will be observed that in scarcely any case does the absorption ratio approximate to unity, although having regard to the fact that thin slices of tissue were used and that the salts employed have fairly high coefficients of diffusion, the equilibrium condition could not be far off at the end of the experiment. This is also indicated by the value of the absorption ratio at the end of, say, four days, being practically identical with that at the end of six days in the case of the absorption of sodium nitrate by *Dahlia* tuber.

It can therefore scarcely be argued that as all these absorption ratios are less than unity the reason for this is that equilibrium had not been reached when the experiments were brought to a conclusion. Moreover, in the case of the absorption of aluminium sulphate by carrot and other tissues Meurer found so great absorption of the aluminium ion from a 0·056 per cent. solution that the absorption ratio with carrot after two days was 11·33 and after four days 16·89. Of course, aluminium may be a special case, and Meurer himself thought it was absorbed by the cell walls. However, as we shall see shortly, there is no reason from the high value of the absorption ratio to suppose that the behaviour of aluminium is exceptional.

In this connexion the results obtained with dyes are interesting. Reference has already been made to the work of Pfeffer and others who have shown that in the absorption of many dyes there is a considerable "heaping up" of the dye in the tissue. This is chiefly, though not exclusively, a property of the so-called basic dyes, and Collander (1921) has shown that a number of sulphonic acid dyes are only absorbed to a comparatively small extent by many plant tissues. Some of the absorption ratios found by him are collected in the following table.

TABLE XXXVI

Absorption Ratios of Sulphonic Acid Dyes.

(Data from Collander)

Species	Tissue	Dye	External concentration in per cent.	Duration of experiment in hours	Absorption ratio
Allium Cepa	Parenchyma of bulb scale	Light green	0·8	46	<0·016
Daucus Carota	Root parenchyma	Orange G	0·8	42	<0·016
Hyacinthus orientalis	Cortex of root	Cyanol	0·1	24	<0·125
,,	Storage cells of bulb scales	,,	0·1	24	<0·125
,,	Parenchyma of peduncle	,,	0·1	24	<0·062
,,	,, ,,	Orange G	0·8	72	<0·0078
,,	Bundle sheath of perianth leaves	,,	0·012	24	>4·0
,,	,,	,,	0·05	24	<0·25
Pisum sativum	Cortex of root	Cyanol	0·8	24	<0·062
Rhœo discolor	Spongy parenchyma	Orange G	0·8	48	<0·062
Spirogyra sp.	—	Cyanol	0·8	50	<0·062
,,	—	Orange G	0·8	90	<0·031
,,	—	Fuchsin S	0·8	66	<0·125

It is clear then that the absorption ratios of dyes may vary within very wide limits, from many times unity in the case of most basic dyes, to less than 0·01 in the case of some tissues immersed in solutions of acid dyes.

With dead tissue the absorption ratios in the case of salts are much nearer unity (cf. Table XXXI). The maintenance of equilibrium with different concentrations inside and outside the cell is thus a property of living tissue.

It is interesting to note that the maintenance of an equilibrium in which the concentration of a substance inside the tissue is apparently different from that outside has also been observed in the case of seeds. Brown and Tinker (1916 b) soaked grains of barley in solutions of aniline, phenol and acetic acid, and determined the extent of the absorption of these substances by analysis of the seeds. They found that aniline and phenol accumulated inside the seeds so that the concentration of these substances inside the seeds was about three times as great as the external concentration. With acetic acid, on the other hand, equilibrium was attained when the concentration of the acid inside the seed was about 80 per cent. of the external concentration, the latter being in the neighbourhood of 40 per cent. (the ratio acetic acid : water being between 0·5 and 0·9).

The influence of external concentration on the position of equilibrium.
The observations already recorded suggest that the quantity of
substance absorbed by the same tissue from a solution is *relatively*
greater the diluter the solution, that is, the absorption ratio increases
with dilution. The influence of the concentration of a solution ex-
ternal to plant tissue on the position of the equilibrium attained in
the intake of a number of salts has been investigated by Stiles and
Kidd (1919 *a*) by means of the measurement of the electrical con-
ductivity of the external solution. Experiments were carried out
at constant temperature (20° C.) with carrot root and potato tuber
in the form of circular disks 1·8 cms. in diameter and 1 mm. in thick-
ness, 40 such disks being immersed in 100 c.c. of solution. These
experiments were all conducted in triplicate. For any series of
experiments designed to yield comparable results all the disks used
were cut at the same time and allowed to swell in water for a pre-
liminary period. After mixing them well together each set of 40 disks
was then taken from the general stock. The bottles used to contain
the tissue and solutions were continuously shaken throughout the
whole course of the experiment. This is a precaution to ensure regular
results, as such treatment prevents the formation in the solutions
of gentle diffusion gradients which may be unequal over different
disks, especially when some of these lie on top of others.

It has been pointed out by Stiles and Kidd that the decrease in
the conductivity of the external solution may be assumed to be
approximately proportional to the difference between absorption of
the salt and exosmosis from the tissue. If the exosmosis into distilled
water is determined and added to the values found in the experiments
with salts, numbers proportional to the actual salt intake should
be obtained provided there are no complications. That this is so
cannot be assumed.

Stiles and Kidd pointed out that the following actions would also
bring about a decrease in conductivity and so make the values
obtained for absorption in the way indicated, too high.

(1) Reactions between the exudate and external solution by
which non-ionised molecules are produced. This can be disregarded
as a source of appreciable error having regard to the dilution of some
of the solutions and the magnitude of the decreases with higher
concentrations.

(2) The exosmosis of non-electrolytes which by their mere presence
would reduce the conductivity of the external solutions. The quantity
of such substance which diffuses from the tissue is probably negligible.

(3) A lessening of exosmosis as compared with the control on account of the action of the salt on the tissue. In the case of carrot exosmosis into distilled water is small, and if the action of the salt were to reduce it to nothing the results would be affected very little.

Hence the method is not likely to give too high values for absorption. On the other hand the following actions would tend to increase the conductivity and so render the observed measures of the absorption too low.

(1) Increased exosmosis resulting from the action of the salt on the tissues. This, long continued, leads to the death of the tissue (Stiles and Jørgensen, 1917 a) and consequently to loss of its turgidity. But at the end of the experiments there was no loss of turgor of the tissues employed, so that it is unlikely the values obtained for absorption are appreciably raised on this account.

(2) An unequal absorption of the ions of a salt as described earlier in this chapter. In this event it is the approximate absorption of the less absorbed ion that would be measured, for the excess of the more absorbed ion must be replaced by an equivalent quantity of a similarly charged ion, either H or OH from the water of the external solution, or by an ion escaping from the tissue, probably the latter (Redfern, 1922 a). Moreover, as the mobilities of the absorbed and replacing ions will be different in all probability, and as a difference in the degree of dissociation may result, the fall in conductivity will only give an approximate value of the absorption. Owing to the possibility of this complication the results of Stiles and Kidd are therefore to be regarded as giving approximate values of the absorption of the less absorbed ions of salts.

In the following table are shown the absorption ratios obtained for the absorption by carrot tissue of various chlorides from solutions possessing initially concentrations ranging from 0·1 N to 0·0002 N.

The results with each salt are all strictly comparable, but the results with different salts should not be so compared as different batches of tissue were used for the experiments with each salt.

If the logarithms of the final external concentrations are plotted against the logarithms of the final internal concentrations the points in the case of each salt lie approximately on straight lines. In Fig. 13 the logarithms of the final external concentrations are taken as ordinates and the logarithms of the final external concentrations as abscissæ. It will be observed that the relation is approximately a linear one in the case of each salt.

TABLE XXXVII

Absorption Ratios in the Intake of Various Chlorides by Carrot
Tissue from Solutions of Different Concentrations.
(Data from Stiles and Kidd)

Salt	Initial concentration in normalities	Duration of experiment in hours	Relative final conc. Internal	Relative final conc. External	Absorption ratio
Potassium chloride	0·0002	52	0·60	0·024	25·0
,,	0·002	,,	4·20	0·238	17·6
,,	0·02	,,	11·90	4·882	2·4
,,	0·1	,,	20·50	26·200	0·78
Sodium chloride	0·0002	48	0·56	0·012	46·7
,,	0·002	,,	4·00	0·148	27·0
,,	0·02	,,	10·80	3·990	3·5
,, (dead tissue)	0·02	,,	4·45	5·060	0·88
,,	0·1	,,	17·80	21·550	0·83
Calcium chloride	0·0002	42·5	0·46	0·030	15·3
,,	0·002	,,	1·17	0·420	2·8
,,	0·02	,,	2·50	4·930	0·51
,,	0·1	,,	5·30	22·120	0·24
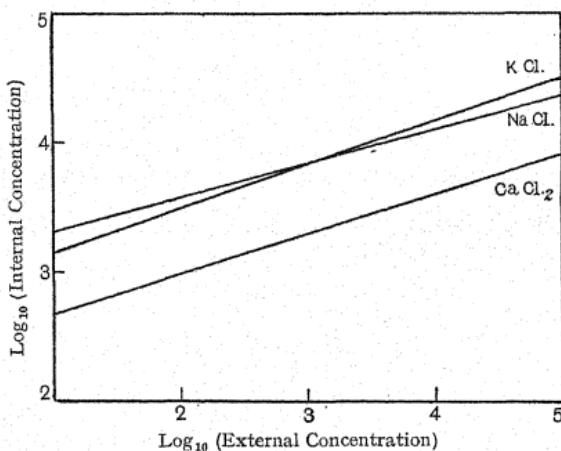
Fig. 13. The relation between final internal and final external concentration
in the case of carrot root tissue immersed in certain chlorides. (After
Stiles and Kidd.)

Then, if i and e represent the final internal and final external
concentrations respectively, the relation between the two is given
by the equation

$$\log i - m \log e = \log k,$$

where $\log k$ is a constant.

This equation may be written in the form $i = ke^m$, and this is the adsorption equation. (See Chapter III.)

Although this result does not prove that the intake of salts into the cell is a process of adsorption of the salt by a constituent of the cell, it indicates that this is a possibility. But the results do give clear proof that the absorption of salt by carrot root does not proceed until there is equality of concentration inside and outside the cell. With lower concentrations there is heaping up of salt inside the cell, while with higher concentrations the absorption ratio is less than unity. That this is connected with the living condition of the cells is evident from the fact that with dead tissue the absorption ratio approximates to unity as shown by Meurer's results already cited, and by the result obtained with dead carrot tissue in 0·02 N sodium chloride by Stiles and Kidd. The absorption ratio found with such dead tissue was 0·88, whereas living tissue under otherwise exactly similar conditions yielded an absorption ratio of 3·5.

This work has been extended by Miss Redfern (1922 *b*) to the uptake of dyes, the same tissue, carrot root, being for the most part employed. The dyes used were neutral red, methylene blue, methyl violet, aniline blue, eosin and congo red. The absorption ratios found with these dyes presented in various concentrations (initially 0·1, 0·05, 0·01 and 0·005 per cent.) are shown in Table XXXVIII.

TABLE XXXVIII

Absorption ratio at equilibrium in the absorption of various dyes by disks of storage tissue. (Data from Redfern)

Dye	Character of dye	Concentration at equilibrium		Absorption ratio
		External	Internal	
Neutral red	Basic; semi-colloid	0·06	2·0	33·3
		0·03	1·0	33·3
		0·0005	0·475	950
		0·000125	0·244	1952
Methylene blue	Basic; crystalloid	0·032	3·4	106
		0·01	1·9	190
		0·0008	0·46	575
		0·0003	0·235	783
Methyl violet	Basic; semi-colloid	0·03	3·5	117
		0·01	2·0	200
		0·0018	0·41	228
		0·001	0·20	200
Aniline blue	Basic; colloid	0·1	—	—
		0·045	0·25	5·56
		0·008	0·1	12·5
		0·003	0·1	33·3
Eosin	Acid; crystalloid	0·1	—	—
		0·045	0·25	5·56
		0·0085	0·075	8·82
		0·004	0·05	12·5

A similar influence of concentration of the dye was found with potato immersed in neutral red solutions, with artichoke tuber in solutions of methylene blue and with turnip root in solutions of methyl violet. It will be observed that the absorption ratios are very much higher in the case of basic crystalloidal and semi-colloid dyes than in the case of the acid eosin and the colloidal, although basic, aniline blue, although in both cases the influence of dilution of the dye is to bring about an increase in the absorption ratio. Miss Redfern has shown that the absorption here also represents approximately the relation between concentration and quantity absorbed at equilibrium. Deviations from this rule may be partly due to the approximate character of determinations by the colorimetric method, and partly to the complicated nature of the absorption process.

The dependence of the position of equilibrium on the nature of the absorbed substance. We have already noted in the case of dyes that the intake of a dye by plant tissue depends very greatly on the nature of the dye. In general basic dyes are absorbed to a very much greater extent than acid dyes, although this rule is not without exception, while among each group of dyes there is considerable range of variation in the extent of intake. Stiles and Kidd (1919 *b*) have shown the same to be the case with salts. In Table XXXIX are shown the absorption ratios they found in the absorption by carrot tissues from solutions of a number of chlorides, sulphates, nitrates and potassium salts in initially the same equivalent concentration, namely, 0·02 N. The results within each group are strictly comparable.

TABLE XXXIX

Absorption Ratios of a Number of Chlorides, Sulphates, Nitrates and Potassium Salts presented to Carrot Tissues in a Concentration of 0·02 N

Group	Salt	Duration of experiment in hours	Absorption ratio
I	Potassium chloride	91	3·58
	Sodium chloride	,,	3·49
	Lithium chloride	,,	1·16
	Calcium chloride	,,	1·09
II	Potassium sulphate	64·5	0·51
	Sodium sulphate	,,	0·46
	Magnesium sulphate	,,	0·097
III	Potassium nitrate	71·5	4·65
	Sodium nitrate	,,	3·30
	Calcium nitrate	,,	1·19
	Aluminium nitrate	,,	0·53
IV	Potassium chloride	42	1·99
	Potassium sulphate	,,	0·55
	Potassium nitrate	,,	2·20

These results show clearly that the extent to which a salt is absorbed by the particular tissue used depends both on the kation and anion of the salt. Salts having the same anion are absorbed in the order K, Na, Li, [Ca, Mg], Al, while salts containing the same kation (K) are absorbed in the order NO_3, Cl, SO_4. This means, as far as these results go, that salts containing two univalent ions are absorbed much more rapidly than salts containing a divalent or trivalent ion. The difference in the position of equilibrium of K, Na, and Li salts on the one hand, and of Ca on the other, is very striking, and so is the difference in the position of the equilibrium between chlorides and nitrates on the one side and sulphates on the other.

The influence of the thickness of the tissue on the position of equilibrium. The only experiments of which I am aware dealing with this question are those of Ruhland (1909 *b*), who compared the absorption by equal weights of disks of beetroot 3 mm. in thickness immersed in 0·4 per cent. calcium chloride, and of carrot disks of the same two thicknesses in 1 per cent. ammonium nitrate. His results are shown in Table XL.

TABLE XL

Influence of the Thickness of the Tissue on the Absorption Ratio

Tissue	Salt	Initial concentration in per cent.	Duration of expt. in days	Thickness of tissue in mm.	Absorption ratio Kation	Anion
Carrot	Ammonium nitrate	1	7	3	0·5276	—
,,	,,	,,	,,	1	0·8342	—
Beetroot	Calcium chloride	0·4	2	3	0·2582	0·0354
,,	,,	,,	,,	1	0·3421	0·0522
,,	,,	,,	4	3	0·3266	0·0486
,,	,,	,,	,,	1	0·5616	0·0826

It is clear that in these experiments the extent of absorption by an equal weight of tissue was considerably increased by increasing the surface of the tissue directly exposed to the solution. While this result is understandable on the view that the absorption of the salts examined is controlled by adsorption, yet the results are so few that it would be premature to elaborate an explanation of the results. Clearly the question is deserving of further examination.

THE COURSE OF ABSORPTION OF DISSOLVED SUBSTANCES

The course of absorption of a number of salts by storage tissues (carrot root and potato tuber) at a fixed temperature of 20° C. has been determined by Stiles and Kidd (1919 *a*, *b*) by the electrical

conductivity method already described. These investigations yielded
information on (1) the influence of concentration on the rate of intake
of salts, (2) the dependence of the rate of absorption on the nature
of the salt, and (3) the course of absorption in general. Some informa-
tion on these questions and on the influence of temperature, light and
wounding on the absorption of dissolved substances is also available
from other sources.

Influence of concentration. This was investigated for the case of
a number of chlorides; namely, those of potassium, sodium and
calcium, the salts being used in concentrations of 0·1 N, 0·02 N,
0·002 N and 0·0002 N in each case. The data obtained with each salt
are all strictly comparable with each other, the disks of tissue
employed being all from one batch.

The actual measurements obtained in the case of carrot are shown
for the three salts in Table XLI and the results for potassium chloride
are recorded graphically in Fig. 14, where the relative absorption
has been assumed proportional to the sum of the fall in conductivity
of the solution and the rise in conductivity of the same quantity of
distilled water containing the same quantity of the same tissue.

TABLE XLI

Changes in Electrical Conductivity of Solutions of Various Chlorides
of Different Concentrations containing Carrot Tissue (40 Disks of
Carrot, 1·8 cm. in diameter and 1 mm. thick, immersed in 100 c.c. of
each Solution). (Data from Stiles and Kidd)

Salt	Time in hours	Distilled water	0·0002 N	0·002 N	0·02 N	0·1 N
Potassium chloride	0·5	—	—	− 3	− 167	~ 610
	6·0	+ 80	+ 58	− 48	− 372	~ 970
	24·0	+145	+ 92	−196	− 892	−1600
	52·0	+196	+137	−223	− 992	−1850
Sodium chloride	3·0	+ 36	+ 30	+ 18	− 113	− 560
	34·0	+ 87	+ 57	−124	− 476	−1070
	41·5	+ 66	+ 19	−287	− 885	−1580
	48·0	+ 58	+ 2	−340	−1020	−1720
Calcium chloride	0·5	—	—	+ 3	− 71	~ 343
	14·5	+ 64	+ 35	− 53	− 145	~ 457
	20·5	+ 86	+ 53	− 57	− 125	~ 370
	36·25	+ 60	+ 17	−105	− 181	~ 503
	42·5	+ 54	+ 8	−116	− 195	~ 470

It is very clear from these tables and figures that the rate of
absorption of salt is dependent on the concentration of the salt, the
greater the concentration the more rapid the absorption. Similar

results were obtained by Miss Redfern (1922 *b*) with dyes. This does not mean, of course, that the rate of equilibration is more rapid in the case of stronger solutions, for a greater quantity of salt is absorbed.

The influence of concentration on the absorption of aniline dyes was examined by Szücs (1910). His method is not ideal. Filaments of *Spirogyra* were placed in solutions of methyl violet of different concentrations varying from 0·000125 to 0·00125 per cent. and the time determined that had to elapse for the cells to acquire a standard depth of tint. It was found that the product of time and concentration was a constant over this range of concentrations, from which
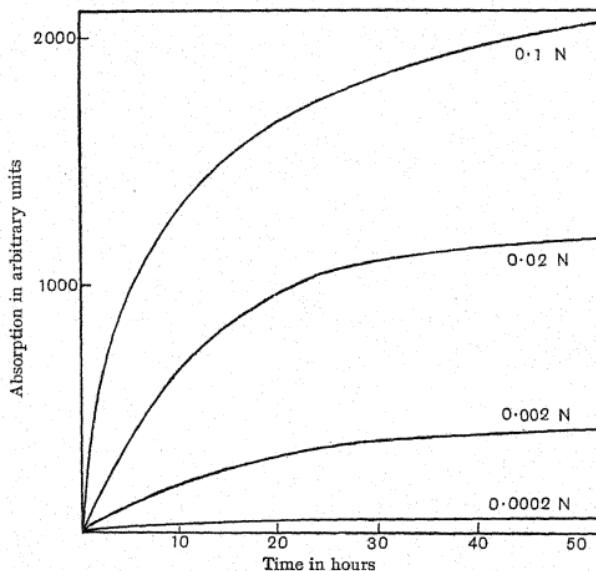
Fig. 14. Absorption of potassium chloride by carrot root tissue immersed in solutions of various concentrations. (From the data of Stiles and Kidd.)

it was concluded that Fick's law holds for the absorption of the dye. It will be remembered that Fick's law states that

$$dQ = - DA \frac{\partial C}{\partial x} dt,$$

where dQ is the quantity of substance diffusing through a cross-section of area A in the time dt, D being the coefficient of diffusion and

$\dfrac{\partial C}{\partial x}$ the concentration gradient in the line of flow. Szücs assumes that the concentration gradient remains constant in each case throughout the time of the experiment, as the concentration of the external medium remains practically the same, and the dye is bound in the cell in some osmotically inactive form. Hence in the case of each concentration the quantity of dye absorbed will be proportional to the time and hence

$$Q = kCt$$

where Q is the total quantity of dye absorbed in the time t when the concentration of the dye in the external medium is C, and k is a constant depending on the coefficient of diffusion and the dimensions of the cells, presumably assumed equal in all cases.

Since Q is the same in all the experiments, it follows that if Fick's law holds, Ct must be a constant. Experiments with neutral red showed that Fick's law does not hold with regard to the absorption of this dye by *Lemna minor*. This is attributed to a complication arising through the adsorption of this dye by the cell wall. According to Ruhland (1908 *a*, *b*) this adsorption is prevented by the presence of hydroxyl-ions, and Szücs found that when neutral red was dissolved in 0·005 N sodium hydroxide, the product of the concentration and time required for the absorption of a definite quantity of dye was a constant both when *Lemna* and *Spirogyra* were used.

The influence of concentration on the rate of intake of two alkaloids (piperidine and quinine) and a purine (caffeine) by two species of *Spirogyra*, was examined by Tröndle (1920) by essentially the same method, the time being measured that was necessary for these substances in various concentrations to produce a visible precipitate in the cells. The same result was obtained as Szücs had found in the case of methyl violet, namely, that the product of concentration and time is constant. This result supports the view that Fick's law of diffusion is followed. This is in marked contrast with the results obtained by Tröndle for the intake of salts, to which reference is made later in this chapter.

The rate of absorption of different substances. Four sets of comparative experiments were carried out by Stiles and Kidd to determine the differences in the rate of intake of a number of (1) chlorides, (2) sulphates, (3) nitrates, and (4) potassium salts. All the salts were employed in the same equivalent concentration, namely, 0·02 N, and the experiments were all conducted at the same temperature of 20° C. The relative absorption of the different salts after different times as

indicated by the change in electrical conductivity is shown in Table XLII for the nitrates examined.

TABLE XLII.

Absorption of Salt by Carrot Tissue from Solutions of Various Nitrates in a Concentration of 0·02 N. (Data from Stiles and Kidd)

Time in hours	Change in conductance of solutions				
	Potassium nitrate	Sodium nitrate	Calcium nitrate	Zinc nitrate	Aluminium nitrate
0·5	– 183	– 89	– 86	– 57	– 20
19·25	– 891	– 659	– 215	+ 107	– 135
71·50	– 2032	– 1360	– 493	+ 540	– 268

These results are in themselves sufficient to show that the initial rate of absorption is dependent on some other factor besides the position of equilibrium. These experiments and those made with chlorides and sulphates indicate that if kations are arranged in order of the initial rate of absorption of salts containing them and a common anion the following series is obtained:

K, [Na, Ca], Li, Mg, Zn, Al,

the relative position of ions placed within square brackets being doubtful. The chief difference between this order and that of the total amount of salt absorbed lies in the position of calcium in the series. Mobility of kation and coefficient of diffusion appear to play a considerable part in determining the initial order of absorption, as might indeed be expected.

TABLE XLIII.

Absorption of Salt by Carrot Tissue from Solutions of Various Potassium Salts in a Concentration of 0·02 N.
(Data from Stiles and Kidd)

Time in hours	Change in conductance of solutions		
	Potassium chloride	Potassium sulphate	Potassium nitrate
0·25	– 145	– 212	– 197
2·25	– 233	– 258	– 214
19·0	– 550	– 266	– 625
42·0	– 1042	– 331	– 1152

The experimental results with three potassium salts are shown in Table XLIII. From these results it appears that the initial order of absorption of anions is

SO_4, NO_3, Cl,
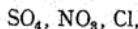
while the final order is NO_3, Cl, SO_4,

the change again being due to the position of the divalent ion, in this case sulphate.

Fitting (1915) and Tröndle (1918 a) have attempted to follow the rate of absorption of salt by measuring the rate of deplasmolysis by their methods described in the last chapter. Fitting concluded that potassium chloride and potassium nitrate penetrate with ease the protoplast of the epidermal cells of *Rhœo discolor*, but that potassium sulphate penetrates much more slowly. Sodium chloride and sodium nitrate both penetrate into the cells, whereas lithium chloride and lithium nitrate enter much more slowly. Magnesium chloride, nitrate, and sulphate only penetrate slowly, while no penetration of calcium and barium salts examined could be observed. The order of absorption found by Fitting for kations was thus

$$[K, Na], Li, Mg, [Ca, Ba],$$

and for anions $[NO_3, Cl], SO_4.$

Later, Fitting (1919) examined the permeability of the same and other cells to glycerol and urea and concluded that urea was absorbed at about the same rate as potassium nitrate or sodium chloride, but that glycerol was absorbed much more rapidly.

Tröndle employed the same method in order to determine the intake of salts by roots of *Lupinus albus* and palisade cells of the leaves of *Acer platanoides* and *Salix babylonica*, and decided that kations were absorbed in the order

$$Rb, K, Li, Mg, Ba, Sr, Ca,$$

and anions in the order NO_3, Cl, $SO_4.$

Kahho (1921 d) investigated the entrance of a number of salts into the young roots of the yellow lupin by means of Lundegårdh's method of tissue contraction and extension described in the last chapter. In order to obtain comparable results with different salts isotonic solutions were used, Fitting's values (1917) of isotonic co-efficients being accepted. Kahho came to the conclusion that the order of absorption of kations is

$$K, Na, Li, Mg, [Ca, Ba],$$

while the order for anions is

$$[Br, I, NO_3], Cl, tartrate, SO_4, citrate.$$
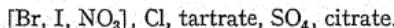
While all these results agree in the main with those of Stiles and Kidd, it is clear from the results obtained by the latter workers that the plasmolytic and tissue extension methods cannot be expected

to give a quantitative measure of absorption. For these latter methods are based on the assumption that after entering the cell the absorbed substance remains in solution in the same condition as it previously existed outside, so that if it were capable of entering the cell it would do so until there were equality of concentration inside and outside the cell, unless the permeability of the cell membranes to the substance became reduced to approximately zero. But as a matter of fact this assumption cannot be correct, for the position of equilibrium depends on the nature and concentration of the substance. Consequently the rate of deplasmolysis does not necessarily give a measure of the total absorption of solute, but only of the increase in the osmotic concentration of the cell sap, which may be a very different thing if the whole of the absorbed solute does not remain in solution and so retain its osmotic activity.

That such a complication is possible was indeed recognised by Fitting (1919), and also by Höfler and Steigler (1921) who investigated the intake of urea by means of Höfler's plasmometric method. Some of the results obtained by Höfler and Steigler may be quoted. They found a tissue, the red-violet epidermis of the stem of *Gentiana Sturmiana*, which absorbs urea with remarkable rapidity. The mean osmotic concentration of these cells lies between 0·4 M and 0·55 M sucrose. When these cells, after a preliminary washing in water for about twenty minutes, are placed in a gram-molecular solution of urea, the intake of urea is such that the concentration of this substance inside the cells increases by about 0·02 to 0·07 gram-molecule per *minute*. This compares with about 0·05 and 0·06 gram-molecule per day found for the intake of this substance by the epidermal cells of the under side of the midrib of the leaf of *Rhœo discolor* by de Vries (1889 *b*); 0·008 to 0·016 gram-molecule per hour found for the same tissue by Fitting (1919); 0·01 to 0·03 gram-molecule per hour by the parenchymatous cells of the internodes of *Tradescantia elongata* immersed in a plasmolysing solution of concentration 0·50 M found by Höfler; and 0·04 to 0·11 gram-molecule per hour found by the same author for the intake of urea by the bulb scales of *Allium Cepa*. If the concentration gradient is taken into account, the absorption of urea by the epidermal cells of the stem of *Gentiana Sturmiana* is thirty times as rapid as in the bulb scales of *Allium Cepa*, forty-five times as rapid as in the case of *Rhœo discolor* and sixty times as rapid as in the case of *Tradescantia elongata*.

This rapid intake is not shown by other substances, for the same cells of *Gentiana Sturmiana* immersed in 0·60 M potassium

nitrate absorb this salt so that (in the mean) the concentration inside the cells increases at the rate of about 0·006 gram-molecule per hour. This is of the same order as the rate found by Fitting and Höfler for *Rhœo discolor* and *Tradescantia elongata* respectively. Thus, we have evidence that the order of intake of different substances is different with different tissues, for in the case of epidermal cells of *Gentiana Sturmiana* urea appears to be absorbed about 170 times as fast as potassium nitrate, but in the epidermal cells of the leaf of *Rhœo discolor* at about the same rate, while Höfler finds for the stem parenchyma cells of *Tradescantia elongata* that potassium nitrate may be absorbed up to five times as fast as urea.

It is also interesting that other cells of *Gentiana Sturmiana* do not show the same high rate of absorption[1] of urea, as the sub-epidermal cortical cells were found to absorb this substance so that its concentration inside the cells increases at the rate of about 0·002 gram-molecules per minute. A similar difference between epidermal and cortical cells was also observed in *Euphrasia Rostkoviana*, *Melampyrum sylvaticum*, *Veronica Beccabunga*, *Homogyne alpina* and *Taraxacum officinale*.

A high rate of uptake of urea, as well as of ethyl alcohol, anti-pyrin and tartaric acid, by the curious hairs extruded from the epidermal cells of the seeds of *Cuphea lanceolata* immersed in water, has been recorded by van Wisselingh (1920), but it is doubtful whether van Wisselingh's experiments were rightly interpreted, for it is not at all certain whether we are here concerned with an action of the living cell or with reactions of a dead constituent of the epidermal cells (cf. Ruhland, 1922).

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[1] Höfler uses the term permeability as practically synonymous with rate of absorption.

(*To be continued*)

CONVOLVULUS NITIDUS BOISS., FROM THE BALKAN PENINSULA

By W. B. TURRILL, M.Sc.

Royal Gardens, Kew

I T is well known that a considerable number of plants are common to the Iberian and Balkan Peninsulas but have not been found in Italy. This is usually, and probably correctly, explained on the basis of the geological history of the countries of the Mediterranean Basin. In the centre of both Spain and the Balkan Peninsula there is a considerable area of ancient land which has probably never been submerged entirely, since, at any one time, at least, the beginning of the Tertiary epoch. Italy, on the other hand, is geologically a young country and owes most of its land surface to foldings and elevations which took place in Miocene and Pliocene times. Hence, it is reasonably concluded that those species of plants which occur in the Iberian and Balkan Peninsulas but miss Italy attained their wide east and west distribution before the Italian peninsula, as we now know it, was formed. Additions to the number of species having the type of distribution just outlined naturally excite considerable interest and the discovery of a species of *Convolvulus*, hitherto known only from Spain, in the mountains of the Balkan Peninsula, is decidedly worthy of record.

Dr N. Stoianoff, Professor of Botany in the Faculty of Agriculture, Sofia University, sent to Kew specimens of a *Convolvulus* under the provisional name of *C. cochlearis*, the specimens having been collected by him in Mt Ali-Botusch. They have been definitely determined by the present writer as *Convolvulus nitidus* Boiss. This species was first described by Boissier, in his *Voyage botanique dans le midi de l'Espagne*, 2, 417 (1839–1847), from specimens collected "in argillosis calcareis aridissimis regionis alpinæ, Sierra Nevada ad Trevenque supra San Geronimo, Dornajo, Aguilones de Dilar. Alt. 6300′–7000′. Fl. Jul." The plant appears to be fairly common in the Sierra Nevada and there are also specimens in the Kew Herbarium from the Sierra del Mana and the Sierra de Segura, both in the south of Spain not far distant from the Sierra Nevada. In Vol. 1, at Table CXXII, of the work cited above, Boissier gives an excellent coloured representation of the plant, accompanied by analyses of the flower.

A morphologically very closely related species is *Convolvulus cochlearis* Griseb., originally described (*Spic. Fl. Rumel.* **2**, 76, 1844) from Eastern Anatolia (legit Donietti). A type-specimen has not been seen by the writer but there seems no doubt from the description that the Greek plants referred to this species by Boissier (*Fl. Or.* **4**, 98) and by Halácsy (*Consp. Fl. Gr.* **2**, 306) are specifically identical with it. The name *Convolvulus parnassicus* was given by Boissier and Orphanides (*Diagn. Ser.* **2**, 3, 125) to the Greek plant, but later this name was reduced by Boissier to a synonym of *C. cochlearis* Griseb. While the majority of specimens from Spain of *C. nitidus* can be easily separated from the Anatolian and Greek *C. cochlearis* by the less compact habit, often longer flowering branches, and larger and longer and less spathulate leaves, there is no doubt that the two are very similar in morphological characters and are presumably closely related phylogenetically. Indeed, part of the material distributed under No. 339 Huter, Porta and Rigo, *ex itinere hispanico* 1870, from Mt Dornajo, Sierra Nevada, can scarcely be distinguished from Greek specimens of *C. cochlearis*. In any case the Ali-Botusch plant is certainly *C. nitidus* Boiss. and not *C. cochlearis* Griseb., whether this latter be regarded as a species distinct from the former or as only a variety of it.

Mt Ali-Botusch, which has yielded many interesting plants to the researches of Prof. Stoianoff, may be regarded as an outlier to the south-west of the great Rhodope massif, itself the home of numerous rare species of plants. The specimens of *Convolvulus nitidus* from the mountain and referred to in this note were collected on July 10th, 1920, between 1800 and 2000 m. altitude, on limestone rocks.
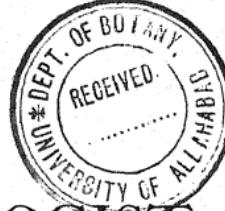
EDITORIAL NOTE

THE TEACHING OF BOTANY

THE following article by Dr F. E. Clements, and another which
we hope to publish later, have been contributed on the invitation
of the Editor. The author was rather in doubt as to the wisdom of
publishing views formed under American educational conditions in
an English journal. Dr Clements's views are, of course, revolutionary
in the strictest sense, and the Editor is far from being prepared to
advocate their full adoption in this country, even if there were any
possibility of such a thing coming to pass! At the same time he is
quite sure that it will be profitable to British teachers of botany to
read, with an open mind, what the author has to say. The Editor is
in full agreement with Dr Clements on at least one point—the extreme
desirability of more experiment, and controlled experiment, in methods
of teaching. The tyranny of tradition is far too strong in teaching,
particularly in university teaching. And it is remarkable that this
should be true in science as much as in other subjects, when we
remember that science owes its life to unfettered observation and
experiment. It is surprising, as Dr Clements points out, that scientific
workers should seldom think of applying the methods of research to
the process of education.

Proposals for the reform of elementary university teaching, which
seemed to their authors rather obviously sound so far as they went,
and at the same time thoroughly workable, were put forward in this
journal some years ago. They were greeted by one distinguished
botanist as "Botanical Bolshevism." It is always desirable to keep
a sense of proportion; and Dr Clements's articles will have served
one good purpose if they demonstrate the difference in this field
between "left wing" idealism and progressive constitutional
Liberalism. We can learn useful lessons from idealism even if we
do not consider its schemes practicable.

THE ECOLOGICAL METHOD IN TEACHING BOTANY

By FREDERIC E. CLEMENTS

A QUARTER of a century ago a young instructor in botany was beginning to question teaching methods at the same time that he was seeking a quantitative basis for ecological research. The endeavour to measure the responses of plants in their habitat suggested the desirability of applying measures to student response. At the outset it was found that a change from the apparently universal practice of announcing examinations beforehand to that of testing the progress of the student by unannounced questions showed a serious discrepancy between supposed and actual accomplishment. In the attempt to remedy this and to make the student's knowledge certain and available at all times, a series of experimental studies was begun which continued until 1917. The essentials of this method of research were summed up as follows in 1911: "I cannot close without pleading that we make the teaching of botany a matter of experiment. We should be ecologists who study the student, the method, the matter, and the results, both as to knowledge and training, in an exact, quantitative manner. If we do this we shall get rid of our loose opinions that for the beginner in botany any method is as good as any other method, and that the results must be good because *we* have done the work. I feel sure that the use of experiment in connection with our methods of teaching, and the measurement of results will go a long way toward changing our present methods and improving our present results[1]."

It is impossible to understand why botanists and scientists generally, who are familiar with the methods and principles of research in their own fields, should never have thought of applying these to the problems of teaching. While tradition and inertia perhaps account for this in some degree, they do not explain why the brilliant advances in science have not been accompanied by corresponding progress in teaching. Some teachers will maintain that they do carry out experiments in teaching, but if published results are to be taken as an index, such experiments are altogether

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[1] *Science*, 33, 645, 1911.

exceptional, as well as desultory and unchecked. Certainly it cannot be gainsaid that the serious faults of current university and college teaching are due to the failure not only to realise that teaching is as proper a subject for investigation as botany, chemistry, or psychology, but also to recognise that it will cease to be a mass of undigested opinions only when the methods and criteria of research are consistently applied to its reconstruction.

It is not the purpose of this paper to deal with the fetishes and traditions that cluster so thickly about university teaching. These are among the most serious obstacles to its progress, since they are so often employed as substitutes for actual thinking about the matter. They are exemplified by the threadbare statements that any method gives satisfactory results in the hands of a good teacher and that the content suited to a special student is equally good for a general one. Even more unfortunate is the custom that demands for the lecture the ablest and most experienced members of the staff, and assigns to the laboratory the newest and most callow of assistants, because of the prestige of the one and the so-called drudgery of the other. While these and other shibboleths are unimportant to the teacher who approaches his problem in the spirit of research, they do serve as touchstones by which to recognise the forward-looking men on whom the burden of making teaching a science must fall.

To those who regard teaching as dependent upon experimental and quantitative investigation, the problem is essentially one in ecological research. This is equivalent to saying that it is a problem involving responses to stimuli, which can be adequately studied only by means of measurement and experiment. The essence of this is the community or group consisting of the teacher and students, in which response, reaction, and correlation can be measured in an environment that also permits of measurement. From the standpoint of the teacher investigation can be directed specially toward environment, methods, content and materials, or results, but each must receive its proper attention in a successful system. While the value of the final product can be determined only by the measurement of results, the desired results can be secured only by the experimental treatment of environment, method, and content. Although the student is much more of an individual than the plant or animal, his individual and community responses are susceptible of fairly accurate study and measurement.

The scrutiny of objectives is the first and perhaps most difficult

task of the teacher who would investigate his own teaching. Since university teachers are practically all specialists, it is not strange that they should have fallen into the almost universal error of teaching their subject rather than the student. Indeed, they rarely teach the subject, but only that fragment in which they are interested. Again, this statement will be sharply challenged by a certain type of teacher, but a glance at his courses will always reveal where his primary interest lies. Until the teacher is convinced that the student is vastly more important than the subject and hence that his interests must be controlling, he is unprepared for an adequate study of his problem. In this connection one of the greatest illusions of the average professor is that facts are unimportant and that only the great principles of his subject are worth being taught. This discloses a curious misconception of the rôle of facts in the development of science, as well as of the principle of recapitulation, in accordance with which the mental development of the individual must reflect that of the race. It is only through facts that the student can find his own way to principles, just as it was done by scientists before him, and it is a sad mistake to assume that this can be discovered for him by the professor. In short, the student must himself be an investigator to whom facts are the indispensable materials with which he builds.

The inquiring teacher will discover that there is but one objective worthy at once of true teaching and of life's opportunities. This is the training of students to be investigators from the outset, and in general courses even more than in special ones, since they afford the only chance of such training for the great majority. He does not need to trouble himself over any fancied difference in the needs of the general and special students in elementary courses, since to acquire the spirit and method of investigation is to give each the most valuable thing possible. Such teaching involves definite and detailed training to observe, to experiment, to think, correlate and apply, and the use of processes and materials to develop interest and knowledge that will be permanent, useful, and usable. It should yield the largest possible human return in the field of botany, but it should go far beyond this, and give human values in insight, vision, and objectivity, that are indispensable to social progress and can be secured in no other way. Science itself can be made to contribute as readily to war and reaction as to peace and progress, and it is only by teaching the research spirit in all things that ethics can be made the ruling force in civilisation.

The basic principle of the process-inquiry system is to make the student as independent of the teacher as companionship in learning will permit. Nothing should be done for the student that he can do for himself, and under the sympathetic encouragement of a research teacher he can do everything that a course should ask him to do. This means that he should have an active share in planning a course that will suit his interests and need, in selecting and organising the processes and materials to be used, and in testing methods and measuring results. Teachers and students should be partners in the business of fashioning the best training possible, and of carrying its values over into everyday life. When this is once fully appreciated, the detailed methods of research teaching and learning readily become evident. Every method that promotes initiative, independence, and insight is to be encouraged, while every one that permits leaning upon the teacher, in the form of lecture, textbook, or notebook, is to be discouraged. The contention that lectures and textbooks are necessary because the student cannot possibly touch all the essentials of a course at first-hand is at fault for several reasons. It assumes that what a student writes in an examination represents mastery and not memory, and also that a set examination furnishes reliable evidence of what he remembers. Moreover, it is based upon an entire ignorance of what students can do where their initiative and independence are encouraged, as well as of the effect of predigested knowledge upon mental fibre. The teacher can quickly demonstrate the relative value of lectures and textbooks by means of unexpected tests of various sorts, of which the written examination is the least valuable. In most elementary and many advanced courses in botany the laboratory notebook belongs properly in a course of drawing, were it not for its indifferent quality. Many notebooks indeed contain nothing but labelled drawings made under the eye of the instructor with much docility but little understanding. Here again it requires but little investigation to demonstrate that what is given to the notebook is taken from the memory, and that a sturdy mental independence is not thus to be secured.

The results obtained by the students working independently are compared and checked at the time by the method of group discussion. In this the teacher takes just as small a part as possible, and the ideal is approached when the group is able to find its own way readily and accurately with but occasional questions or suggestions from him. Group discussions should always be held on the actual spot where the work is being done, whenever the class is

brimming over with results and ideas that require comparison,
checking and correlation. It is all but fatal to postpone discussion
until the glow of inquiry has faded and then to hold it in the ordinary
class-room with stiff rows of seats. Spontaneity and enthusiasm are
invaluable group assets, and they can be obtained in the highest
degree only in actual contact with living plants. This has a further
advantage that differences of opinion can be referred directly back
to the plant for answer. Hence, there should be no distinction between
laboratory and class-room, but all of the work, both individual and
group, should be done where the plants are, whether this be the
greenhouse, garden, field, or (much less satisfactory) the ordinary
laboratory.

The content of elementary courses in botany and other sciences
has practically always been determined by the interest and ex-
perience of the professor in charge, and rarely are the needs and
interests of the students taken into account. Abundant evidence of
this can be discovered during the course itself, but the most con-
clusive testimony is furnished by the complete indifference of the
vast majority of general students to the plant world after the course
is finished, and the gradual extinction, in America at least, of the
botanical amateur. To one that has inherited a course in elementary
botany or has patterned one after the usual models, it seems revo-
lutionary to contend that the students should be given a large or
even a controlling share in determining the content as well as the
methods of such a course. To one who has tried both ways, it seems
incredible that teachers should still continue to feel that they alone
know what is best for their students, and to give them such pabulum
as the differentiation of the stele or alternation of generations. The
practice still current of placing a section of some part of an unknown
and invisible plant under the microscope for the purpose of having
it drawn would be ludicrous, if it were not so wasteful of interest and
opportunity. It is impossible to detail in a brief paper the cumu-
lative evidence brought by students against the elementary course in
which morphology is paramount. Moreover, it is unnecessary to do
so when they are giving a proper share in determining the content,
selecting the materials, and organising the methods of investigation.
In every instance they have chosen to deal with growing plants
familiar in field and garden, and they reach morphology and histology
in the professional sense through the intense interest aroused by the
study of function.

Perhaps the one greatest handicap to effective teaching has been

the lack of methods for ascertaining results. While there is no hope for the teacher who knows that the results are good because they are his, such fortunately are rare, and the great majority will doubtless welcome methods that enable them to know what their results are. Many a professor has admitted that his teaching did not secure tangible results, but has insisted that he did obtain intangible ones, which are much the more important. Likewise it has often been conceded that a year or two of elementary work left the student with little or nothing of value, but stoutly maintained that this was not true of the professional student. In this connection it will suffice to call attention to the general dissatisfaction as to the preparation of young botanists expressed by the heads of scientific bureaus. The actual measurement of results shows that we do no better by professional than by general students, when allowance is once made for their greater incentive and the cumulative effect of advanced courses.

The progressive teacher who would measure the value of his teaching must at the outset divest himself of prepossession, his own as well as that of his best students who especially affect his courses. More than one such student has been known to speak glowingly of the merits of this or that course, only to fumble miserably when asked to give concrete evidence of its value in his own thinking. The teacher may first wish to determine merely the extent to which the students retain the knowledge of the course, and this is readily done by unexpected questions, written or oral, during the term. Much more important is to ascertain what is left of the course after a summer's vacation and after the lapse of a year or more, as it is this alone that can materially affect the student's thinking or his habits. Tests of this are more difficult to apply, except where the student continues with the same teacher. They require cooperation throughout a department or a college, and can be made successfully only when there is general appreciation of the necessity of research in teaching. By far the most important tests are those that have to do with the student's complete progress rather than with the memory primarily. These are termed practical, applied, and correlation tests, and are directed especially to the skill and accuracy with which he can organise his results and apply them to other problems of the same and different categories. With this are associated progress tests of the various learning processes, namely, observing, experimenting, reasoning, remembering, etc., which enable both student and teacher to follow the actual advance in each, and to direct

particular effort to the process that lags. At the outset it is necessary to determine the performance of each student in each process, and this is used as the basis for measuring the progress during the course.

In conclusion it may be desirable to summarise the functions of the teacher as worked out in actual experience with the process-inquiry system. He guides the student from the first day to do his own planning, observing, experimenting, thinking, and applying, in the fullest appreciation of the student's interest in life rather than in the dead and often "pickled" end-results of it. He is a sympathetic and enthusiastic companion in learning, but he does not destroy the student's initiative and responsibility by answering questions or doing anything else that the student can do for himself: He recognises that learning is life, and that the student must be allowed to make and correct his own blunders, since he learns most certainly and permanently in this way. He realises that the growing ability to plan, observe, experiment, reason, and apply results is the only real test of progress, and he refuses to regard the usual classification of students on the basis of set examinations as either fundamental or final. Finally, he is convinced that teaching is as much a subject for experiment and measurement as any science, and that the teacher must be an ardent inquirer as well as the student.

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HYBRIDISM IN THE NEW ZEALAND FLORA

By L. COCKAYNE

GENERAL

AT the present time, when Mendelian methods dominate genetic research, and when an assertion that a plant is of hybrid origin will not be definitely accepted unless supported by properly-conducted breeding experiments, field-observations are either ignored, or take a quite minor place. Nevertheless, have not such observations an honourable place in science? Are they not indeed not only a necessary preliminary for future genetic studies, but should they not teach much that is fairly conclusive regarding the causes of the polymorphy so common in a wild plant-population among those groups of individuals recognised in floras as "species" and "varieties"? In fact, for many years to come, since breeding methods usually demand much time, must not field-observations be relied upon to decide, for the time being, many critical cases concerning the status of so-called "species"? Therefore, in this paper are brought together the results of many year's personal experience in the field, so far as hybridism in the New Zealand flora is concerned.

By those interested in the wider aspects of floristic and ecological research the flora and the vegetation of New Zealand are held to supply material of peculiar importance. This belief is based partly on the extreme isolation of an area with its land-surface, though offering climatic and edaphic conditions of many kinds, not too large to forbid detailed study; and partly on the diverse composition of the somewhat small flora with its palæozelandic, Australian, palæo-tropic, subantarctic and subcosmopolitan elements[1].

The time seems ripe for the publication of information as to the natural occurrence of hybrids. Genetic studies have reached a fairly high degree of intensity. Lotsy's suggestive theory of hybridisation in regard to evolution[2] cannot be lightly dismissed. The part played by indigenous hybrids in floras demands increased investigation in the field. It is high time, indeed, that a comprehensive study of the

[1] For details regarding these elements see L. Cockayne, *The Vegetation of New Zealand (Die Vegetation der Erde*, Bd. 13), pp. 314–323, Leipzig and New York, 1921. The term "subcosmopolitan" is here substituted for "cosmopolitan."

[2] *Evolution by means of Hybridization.* The Hague, 1916.

wild hybrids of the earth should be made and its results replace the
excellent but now obsolete monograph of Focke[1].

It might well be expected that the floras of different countries
would contain descriptions of the hybrids as well as of the species,
but this is far from being the case, the majority of such works having
little, or nothing, to say on the subject. There has existed a feeling
amongst taxonomists—nor is that feeling extinct by any means—
that either wild hybrids are so few as to be negligible, or that they
are of a low status hardly worth mentioning. Indeed certain botanists
have doubted their existence altogether[2]. Few, apparently, have
recognised that, as a flora is essentially an instrument for the identi-
fication of *any plant growing wild in the area with which it deals*,
hybrids should bear names and be described equally with species.
This is the regular procedure in *Das Pflanzenreich*, Grosser, for in-
stance, devoting several pages to *Cistus* hybrids[3], and even supplying
a key for their ready identification. Also, certain monographs do
not neglect hybrids, *e.g.* to mention two of importance to the New
Zealand flora, those of Haussknecht[4] and Bitter[5].

Unfortunately, the conclusions arrived at concerning hybrid
origins are too frequently derived from the study of dried plants,
whereas no really legitimate dictum can be made except one based
on field-observations, since such alone can declare to what extent
any suspected hybrid fulfils those conditions which, in lieu of genetic
research, can decide, almost for certain, whether the plant in question
be a hybrid. The following seem the principal conditions[6] and it is
upon the fulfilment of these, subject to the exceptions cited below,

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[1] *Die Pflanzenmischlinge.* Berlin, 1881.
[2] For example, C. B. Clarke in *Journ. of Bot.* p. 228, 1891, when criticising
Haussknecht's action in describing hybrids of *Epilobium*.
[3] *Cistaceæ*, Heft 14 (4, 193), pp. 27–32. Leipzig, 1903.
[4] *Monographie der Gattung Epilobium.* Jena, 1884.
[5] Die Gattung Acæna. *Bibliotheca Botanica*, Heft 74, I, II, III, IV. Stuttgart,
1910–11.
[6] See L. Diels, Die Methoden der Phytographie und der Systematik der
Pflanzen, *Handb. d. biolog. Arbeitsmethoden*, Abt. 11, Teil 1, Heft 2, pp. 169–70.
Berlin, 1921. Diels gives in addition to those below, (1) the variation is often
of a degenerate or monstrous character, (2) the pollen grains are frequently
misshapen or the fruits badly developed or wanting; (3) their distribution is
irregular and the hybrid frequently absent; (4) compared with the parents
they are few in number.
Also, see R. A. Rolfe, Hybridisation viewed from the standpoint of Syste-
matic Botany, *Journ. R. Hort. Soc.* (Hybrid Conference Rep.), **24**, p. 199, 1900.
Rolfe's conditions are much the same as mine. He also states that, "sometimes
the influence of one parent preponderates to such an extent that it becomes
difficult to identify the second one"—"some individuals derived from the same
two species being so dissimilar as to have been at first considered essentially
distinct in their origin."

that my belief in the hybrid nature of certain of the groups of individuals represented in the list at the end of this paper is based.

1. The alleged hybrids should be more or less intermediate in character between the reputed parents, or, better still, there may be a graduated series of forms leading from one parent to the other. 2. The reputed parents and their hybrids should grow in fairly close proximity. 3. The alleged hybrid, if fertile, should give rise to a more or less polymorphic progeny.

Certainly some of these conditions, and those in footnote 6, p. 106, may not be fulfilled and yet hybridism be almost certain. Thus a hybrid may be more or less invariable; it may breed true, or produce offspring but little polymorphic; as a result of change in an association hybrid individuals of considerable age may occur, even if one or both parents are absent[1], or the parents may be present and the hybrid wanting; in short, there are no absolute criteria, so each case of suspected hybridism must be decided on its merits.

With regard to the application of the above principles in the field, it is clear that no absolutely conclusive proof can be given that any group of plants sexually reproduces itself true. Nevertheless, if a certain group, consisting of similar individuals differing only slightly according to fluctuating variation, is found in several—perhaps many—localities, it seems highly probable that the group in question is a pure strain. To be sure, such a group may vary greatly when in some habitat differing considerably from that where it usually occurs, but such environmental changes are constant characters, and their nature is recognisable in most cases. Such a group may be connected with a related group by intermediates, but only if both groups grow in close proximity.

"Intermediates," such as those just mentioned, are considered in many floras an indubitable proof that the groups thus connected are not true-breeding entities, but that they "vary" and "run into" one another; therefore, such groups must be united, indeed, they are considered one and the same. For such an assumption, in the light of genetic studies, and even of field-observations, there seems not the slightest proof. The intermediates occur *only* when individuals of both groups grow near one another, otherwise there is no "varia-

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[1] Thus Kerner (*The Natural History of Plants*, translated and edited by F. W. Oliver, London, 1895, Vol. II, p. 592), writes of explaining "the phenomenon that species which, from their characteristics, may be looked upon as hybrids of two other species, occupy in each case a district which is separated, and often at a considerable distance, from the areas inhabited by the species supposed to be their progenitors," and specific cases are cited.

tion." Hybridism, a known cause of similar variation, is certainly
a more scientific explanation than is the bald statement that the
group is "variable," and yet no *cause* assigned for such variation,
except the unproved and unlikely supposition that an organism has
within itself some factor which can cause it to produce such inter-
mediate offspring. Even, if mutations were taking place far more
frequently than has ever been suggested, their nature is not con-
sidered that of forms intermediate between distinct groups, their
distinguishing feature being the possession of one, or more, *new*
characters and of breeding true. So in what follows, when in the
Manual[1] it is stated, that two groups of individuals, to which specific
rank had previously been assigned, could not be considered species
because they were connected by intermediates, to me it is good
evidence that such groups are true species, and the intermediates
between them hybrids! And, in nearly every instance this opinion
has been supported by field-observations.

Darwin in *The Origin of Species* makes a distinction between
"mongrels" (crosses between varieties) and "hybrids" (crosses be-
tween species), but he considered that, except in the matter of
fertility (mongrels being usually fertile), there was "the closest
general resemblance" (sixth edition, p. 247) between them. Re-
garding this matter of fertility Darwin had animals chiefly in mind
and was comparing the domestic with the feral. But, in the case of
plants, the matter is different for, as every gardener knows, there
are hundreds of self-fertile hybrids, and some breed true.

As for the species and varieties of floras generally no distinction
can be drawn between them, for what is considered a variety one
day may be acknowledged as a species the next and *vice versa*. Nor,
in the light of present-day knowledge, can any distinction be made.
There is the aggregate species—a quite arbitrary group—its content
being a matter of opinion and not of natural law. The true-breeding
groups of individuals (the "jordanons" of Lotsy, *l.c.* p. 27) are the
realities, the aggregate species is merely a *convenient abstraction*[2].
When there is a group of individuals not too closely related to any
other group it is placed by itself and may be termed an "*invariable*

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[1] T. F. Cheeseman, *The Manual of the New Zealand Flora*, Wellington,
1906. Throughout this paper cited as *Manual*. A new edition is being prepared
by Mr Cheeseman.

[2] For a recent discussion of the species question see L. Diels, *l.c.* pp. 161–65;
also Lotsy, *l.c.* pp. 13–28. For the full reasons for my views on the subject
see A Consideration of the Terms "Species" and "Variety" as used in Botany,
with Special Reference to the Flora of New Zealand, *Trans. N.Z.Inst.* **49**,
pp. 66–79, 1917.

species." Obviously such a species may be either a relict, or it may be apparently of recent origin[1] with no near relatives; nevertheless, it is really biologically equivalent to any of the varieties (jordanons) of an aggregate species, and should all such, except one variety, die out in course of time, the surviving group would be a variety no longer but an invariable species (the monospecific linneon of Lotsy, *l.c.* p. 157), see Cockayne, *l.c.* p. 76.

Notwithstanding the opinions expressed in the last paragraph, for the sake of comparison with the usual floristic treatment of hybrids, I am separating those of New Zealand into (1) *hybrids between species* and (2) *hybrids between the varieties within an aggregate species.* Naturally the former are of the more general interest, and so receive the chief consideration. The matter of hybrids between varieties within aggregate species is chiefly concerned with the important question of the so-called "intermediates" and polymorphy of species in general. Only the spermophytes are dealt with. The literature relating to New Zealand *wild* hybrids is trifling; any of moment is cited further on.

In concluding these general remarks I wish to thank most sincerely the following botanists who have rendered valuable assistance: Mr E. Atkinson (Dept. of Agriculture, N.Z.), Mr. H. H. Allan, M.A., F.L.S. (Feilding, N.Z.), Mr B. C. Aston, F.I.C., F.N.Z.Inst. (Wellington, N.Z.), Mr C. E. Foweraker, M.A., F.L.S. (Canterbury College, N.Z.), Prof. R. M. Harper (College Point, N.Y., U.S.A.), Prof. J. W. Harshberger (University of Pennsylvania), Prof. Aug. Henry (Royal College of Science, Dublin), Prof. W. L. Jepson (University of California), Mr R. M. Laing, B.Sc., F.N.Z.Inst. (Christchurch, N.Z.) and Mr A. W. Page, M.A. (Christchurch, N.Z.).

HYBRIDS BETWEEN SPECIES

General. Under this head are included all suggested hybrids in which aggregate and invariable species (one or both) are concerned. In the case of crosses between two invariable species it is assumed that the actual parentage is evident; but, in the case of crosses where one or both parents are aggregates, unless the actual parental variety is known, the true parentage of the hybrid lies in doubt. At any rate, more distinct hybrid forms may be expected in the latter case than in the former and certain genera should be specially affected, *e.g. Acæna, Celmisia, Epilobium* and *Veronica.* Crosses will also occur between the hybrids themselves, or between these and one or

[1] See J. C. Willis, *Age and Area*, pp. 216–17. Cambridge, 1922.

other of the varieties (not of necessity the parental one), while self-pollinated and inter-varietal hybrids will add more diversity. In this way a medley of forms may arise, whose history cannot possibly be guessed, and which might defy genetic research.

The total number of possible hybrids given in the list at the end of this paper is 128. This number is certainly too small, even if considerable allowance be made for the inclusion of doubtful cases. The families affected number 33 (30 per cent. of the spermophyte families of the flora) and the genera 55 (16 per cent.) of which four are endemic. The only families and genera containing any considerable number of hybrids are: *Compositæ* 42, *Scrophulariaceæ* (*Veronica*) 16, *Celmisia* 12, *and Olearia* 11; the three last-named may be considered palæozelandic. For these families and genera the totals are certainly too low, while there are other genera which probably contain more hybrids than given in the list, especially *Uncinia, Clematis, Ranunculus, Pittosporum, Rubus, Acæna, Hoheria, Pimelea, Epilobium, Gaultheria, Dracophyllum, Gentiana, Coprosma* and *Cotula*. Further, the following genera, not in the list, most likely contain a certain number of hybrids: *Potamogeton, Agrostis*[1], *Carex, Juncus, Luzula, Thelymitra, Colobanthus, Cardamine, Carmichælia, Metrosideros, Oremyrrhis, Halorrhagis, Calystegia, Ourisia, Euphrasia, Plantago, Wahlenbergia*[2] and *Craspedia*.

The only flora, rather larger than that of New Zealand but occupying about the same area, of which I have statistics is that of the British Isles. This, according to *The London Catalogue of British Plants* (10th ed. London, 1908) contains 251 hybrids, or nearly twice as many as in my list, but this total includes no less than 59 hybrids of *Salix*, 33 of *Epilobium*, 21 of *Carex*, 18 of *Rubus*, 13 of *Rumex* and 12 each of *Euphrasia* and *Potamogeton*, while the genera concerned are three less than in New Zealand[3].

Coming next to the growth-forms of the New Zealand hybrids the following is the number for each in the comprehensive classes: *trees* 17, *shrubs* 49, *semi-woody plants* 28, *perennial herbs* (in a restricted sense) 18, *grass-like plants* 6, *tussocks* (including *Phormium* and *Astelia*) 4, *woody lianes* 4, *shallow-water plants* 2.

[1] I follow Hitchcock in referring *Deyeuxia Forsteri* to *Agrostis* (*The Grasses of Hawaii*, pp. 149–50, Honolulu, 1922) and I look upon some of the varieties in the *Manual*, pp. 868–69, as species.

[2] N. E. Brown has shown that *W. gracilis* A. D. C. as defined in the *Manual*, p. 402, is a mixture of three species (*Gard. Chron.* **54**, pp. 316, 317, 355).

[3] There is an interesting paper on Natural Hybrids by R. I. Lynch in the Report of the Conference on Genetics (*Journ. R. Hort. Soc.* **31**, pp. 159–67, 1906).

As for distribution the hybrids occur in the following plant-formations to each of which is attached the number found therein: *rain-forest* 25, *subalpine herb-field and fell-field* (taken together) 25, *tussock-grassland* 11, *stony river-bed* (lowland to montane) 10, *sub-alpine-scrub* 7, *coastal scrub* 7, and, in addition, 23 other formations also contain hybrids.

Finally, the altitudinal distribution of the hybrids is: *coastal* 13, *lowland* 34, *subalpine* 39, *lowland and montane* (both) 19, *lowland, montane* and *subalpine* (all three) 2, *montane and subalpine* (both) 21.

An accurate knowledge of the relative distribution of the alleged parental species is of prime importance. This can be conveniently studied in New Zealand proper[1], owing to the comparatively narrow land-surface extending from north to south through nearly fourteen degrees of latitude[2] and the general proximity of lofty mountain ranges to the lowland belt. Thus, speaking of the distribution of the New Zealand flora in general, and commencing at the far northern limit of the North Island, certain species range more or less continuously throughout the whole area, but a large majority have their respective northern and southern limits within the area. This depends, in the case of coastal and lowland species, partly on their relation to frost[3]. Now, amongst the different genera are many related species each with its distinct range; but, at definite points, some of these come together and extend jointly north or south for a certain distance. If the ecological requirements of such pairs of species are fairly equivalent they will, at times, occur side by side, and crossing then becomes possible, its frequency depending upon the time and duration of the flowering season, and their requirements in regard to cross-pollination. The latter has been but little investigated, but, as unisexuality is common and dichogamy apparently frequent, there are often opportunities for crossing.

In certain instances intermediate forms appear in the first locality where the two species come together. The main cause of the

[1] This includes the North Island, the South Island and Stewart Island. The New Zealand Botanical Region also comprises the Kermadec, Chatham and Subantarctic Islands.

[2] Really the 34th parallel of South latitude lies some 20 miles north of the North Cape and the 48th parallel some 30 miles south of the South Cape of Stewart Island.

[3] For a fuller account of plant-distribution in New Zealand see L. Cockayne, *The Distribution of the Vegetation and Flora of New Zealand*, Cawthorn Lecture, Nelson, N.Z., 1921. For the part relating to latitudinal distribution see pp. 12–16.

presence of hybrids, apart from the power of cross-pollination, is
the occurrence of related species side by side. In this relation there
is every transition from species which nearly always grow together
to those which never, or very rarely, do so owing frequently to their
belonging to different altitudinal belts, or plant-associations, or to
their extreme rarity. Another class of hybrids occurs where one of
the parents is confined to some particular, perhaps unusual, habitat
into which the other parent of wider range and ecological capabilities
has penetrated. Or both parents may be confined to a quite small
area, and a particular habitat, where they hybridise so freely that
there is a continuous chain of intermediates between the extremes,
in which case floristic botanists generally, and without hesitation,
define the group as a "variable species." All the above cases refer
to *the primitive plant-covering*. It is the fact of considerable hybridi-
sation taking place in a *virgin vegetation* which is a leading motive
for this paper. But the *effect of settlement* is also an important matter.
It was only some eighty years ago, that agriculture commenced to
modify substantially the natural physiognomy of the landscape, but
during those few years surprising changes have come about. Associa-
tions both of indigenous and introduced plants, and combinations
of the two—some with quite a primitive stamp—have come into
being and occupy wide areas. In these man-induced associations a
few species have found more opportunities for crossing than formerly,
so that their hybrids are now far more numerous than in primeval
New Zealand. To the powerful effect of man in unconsciously
favouring hybridisation Professor Aug. Henry has recently called
my attention in a valued letter which I should have greatly liked to
have published, but, of course, this cannot be done without his per-
mission. It will be seen in some of the cases considered below that
his ideas receive strong support.

Under the next head a brief account is given of certain
hybrids. For sake of brevity little is said about their taxonomic
characters. Where it is stated that the hybrids are "inter-
mediates," it is meant that there is a blending of the parental
specific characters. But many hybrids in a series may possess
the characters both blended, or unchanged, or characters may be
absent.

Special details. The following classification is based upon the
relative opportunity which their distribution (latitudinal, vertical and
ecological) affords for pairs of species to come together. Certainly,
as crossing experiments have shown for plants in general, and even

for those of New Zealand[1], there are many species, now isolated, which would probably hybridise should the opportunity arise[2]. This, in certain cases may be expected to happen at any time through rapid alterations in plant-associations either by natural agencies or the operations of man. Like all classifications the one used here contains some anomalies: also there are certain hybrids which, with equal propriety, might be placed into more than one class.

1. *Hybrids between species of wide range, often belonging to the same associations, which frequently grow in close proximity.*

In this class the opportunity for crossing is at its maximum; the number of its hybrids[3] is 27.

(a) Fuchsia Colensoi × excorticata.

Fuchsia Colensoi is either a straggling shrub or a scrambling liane; *F. excorticata* is a small tree with a rather thick, irregular trunk. Both species are common members of rain-forest; when one alone is present there is no polymorphy. The hybrids form a long series of intermediates between the parents. Damaged forest re-generating increases the number of both species, thus favouring their crossing.

(b) Coprosma propinqua × robusta (= *C. Cunninghamii* Hook. f.).

Coprosma propinqua is an erect, divaricating shrub with narrow, short leaves, and flowers solitary or in few-flowered fascicles; *C. robusta* is a tall stout, bushy shrub with far larger, broader leaves and with its flowers in dense many-flowered glomerules. Both grow together in swamps and swamp-forest. The hybrids exhibit every gradation[4] between those of the parents in inflorescence, leaves and colour of drupes, the last-named being not merely "pink and trans-lucent" as given for *C. Cunninghamii* in the *Manual*, p. 249.

[1] Various hybrids of *Clematis, Fuchsia, Veronica (Hebe)* and *Celmisia* have been purposely raised or have accidentally originated both in European and New Zealand gardens.

[2] An interesting case of this kind was recently published by J. W. Besant in the *Gardeners' Chronicle* (**72**, p. 49 and Fig. 23, p. 52, 1922) which tells of a hybrid which arose naturally in the garden of Sir John Ross of Bladensburg at Rostrevor, Scotland, between the Australian *Olearia argophylla* and the New Zealand *O. macrodonta*.

[3] Unless the contrary be stated none of the hybrids dealt with in this paper have previously been considered such, but have been treated as species or intermediates, or have received no special recognition.

[4] On the magnesian soil of the Mineral Belt (Nelson) grows a shrub, hitherto referred to *C. Cunninghamii*, which is most likely an undescribed species.

(c) Apium filiforme × prostratum.

These two herbs constantly occur together as members of salt-meadow together with intermediates of many forms. In the *Manual*, p. 205, because of such intermediates the species are united, nevertheless *filiforme* is upheld as a variety. Inland *A. filiforme* (*A. prostratum* being absent) shows no polymorphy other than environmental.

(d) Acæna inermis × microphylla.

These species frequently occur side by side on stony ground, especially in the montane belt. The special distinction between the two is the presence and absence of bristles on the flower-heads. In the hybrids all degrees in the production of bristles can be seen on the same individual. There is also a multiplicity of forms—especially colour of leaf—due, doubtless, to crosses between the varieties of the two aggregate species. This case would lend itself admirably to Mendelian analysis.

2. *Hybrids between species of a more or less similar wide range and ecological requirements, but which only meet occasionally.*

This class is the opposite of (1); it includes 13 hybrids. In most cases probably slight ecological differences in their requirements keep the species apart.

(a) Celmisia Lyallii × spectabilis (= *C. pseudo-Lyallii* (Cheesem.) Cockayne). Its hybrid origin is suggested in *The Vegetation of New Zealand*, p. 342, 1921.

This is apparently a case where a more or less invariable hybrid is produced, which, if truly invariable, should be looked upon not as a hybrid but as a valid species which differed only from species in general in that its origin was actually known. The hybrid has not been recorded from localities where one of the parents is absent. It has the leaf-form of *C. Lyallii*, but without the characteristic grooves, and the texture and tomentum of the leaf is that of *C. spectabilis*. In some places the hybrid is abundant.

(b) Celmisia coriacea × Lyallii.

This hybrid, discovered by Mr H. H. Allan on Mount Peel (Canterbury), somewhat resembles that just dealt with in its leaf-form, but it has the silvery tomentum of *C. coriacea*. It is apparently rare. In some respects it is not unlike *C. Petriei* Cheesem.

(c) Muehlenbeckia axillaris × ephedroides (= *M. muricatella* Col.).

This hybrid has been observed in several localities where, on gravelly ground, the two species grow side by side. It preserves the

habit of *M. ephedroides* but is far more leafy. Mr B. C. Aston first suggested to me that it might be a hybrid.

3. *Hybrids between species of different, but considerable, latitudinal range, which, from a definite point, grow in close proximity for a certain distance.*

The number of hybrids in this class is 30; the following are instructive examples.

(a) Melicope simplex × ternata (= *M. Mantellii* Buch.). Accepted by T. Kirk as a hybrid in *The Student's Flora of New Zealand*, p. 86.

Both species belong to rain-forest. *M. simplex* is an erect, twiggy shrub with small 1-foliate leaves and small flowers, 1–3 on each peduncle, and *M. ternata* a small, bushy tree with fair-sized 3-foliate leaves and larger flowers in panicles. The hybrids exhibit every transition in habit, leaf and flower between the two parents. *M. simplex* extends from north to south throughout the lowland belt of both islands, but *M. ternata* halts a few miles to the south of latitude 42°. From the extreme north of the North Island southwards to the above line the hybrids are common, but further south there are none. So, too, on the Kermadec Islands where only *M. ternata* occurs.

(b) Myrtus bullata × obcordata (= *M. Ralphii* T. Kirk). Treated as a hybrid by L. Cockayne in *Trans. N.Z.Inst.*, 50, pp. 179–83, 1918.

Not only was *M. Ralphii* not considered to be of hybrid origin, but in the *Manual*, p. 169, there is no hint of its polymorphy. Nevertheless, as I have shown from an examination of many specimens and observations in different localities, there are transitional forms of every kind including almost pure *M. bullata* and almost pure *M. obcordata*. The former species is common in rain-forest, and its outskirts, throughout the lowland belt of the North Island, but it halts somewhat to the south of latitude 41°; the latter species extends from the south of the South Island almost as far as latitude 35° in the North Island. At the southern limit of the range of *M. bullata* the hybrids suddenly appear and they continue northwards, so far as is known, wherever the two parents come together. Although *M. bullata* is common in the far north of the North Island, *M. obcordata* has been observed only in the *one locality* (Reef Point) which forms its northern limit. Here *M. bullata* is also present and

so is the hybrid. Southwards from this point *M. obcordata* is absent for a distance of about 60 miles.

(c) Corokia buddleioides × Cotoneaster (= *C. Cheesemanii* Carse).

Corokia buddleioides hardly extends to the south of latitude 38°, but *C. Cotoneaster* ranges throughout the entire length of both islands, being commonest in the montane belt. The first-named is an erect, fairly tall bushy shrub and the latter a ball-like, extremely dense, wiry divaricating shrub; also in leaf and flower they differ widely. I have not seen the hybrid in its habitat, but Carse's description[1] seems to establish its origin beyond doubt.

(d) Cassinia fulvida × leptophylla.

Cassinia leptophylla is absent throughout the South Island up to about latitude 42°, but *C. fulvida* is common throughout. Both are very closely related, but its yellow tomentum on the undersurface of the leaves gives the latter a quite different appearance. As soon as the species meet intermediates appear and a mixed assemblage is present which, if it were all that was known of the group, would certainly be considered a "variable species." From their first coming together the species, having crossed Cook Strait, extend in company along the coast through the southern part of the North Island. After the coastal forest is felled, and burnt, these species of Cassinia, and their hybrids, extend some distance inland and form that dense shrubland, the "tauhinu scrub," so dreaded by farmers. Here, then, is a case, as suggested by Professor Henry, of the chance for hybridisation being enormously increased by settlement.

4. *Hybrids between species of wide range and species of comparatively small range, and restricted habitat, which lie in the path of the former.*

There are 11 hybrids in this class some of which might quite well go into class (5).

(a) Gaultheria oppositifolia × rupestris (= *G. fagifolia* Hook. f.).

The aggregate species, *G. rupestris*, extends throughout the South Island and continues its progress through the North Island to the Thames Botanical Subdistrict; it also ascends to above 6000 ft. On the pumice soil of the Volcanic Plateau it comes into contact

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[1] "The plant appears to pass by regular gradations into *C. Cotoneaster* on the one hand and *C. buddleioides* on the other" (see *Trans. N.Z.Inst.* **45**, p. 276, 1913). The description speaks of "the typical form" as "very distinct," but it is difficult to see why any particular form should be more "typical" than any other of these intermediates.

with the distinct *G. oppositifolia*, and at once hybrids appear in plenty. Cheeseman (*Manual*, p. 407) suggests a hybrid origin for *G. fagifolia*, a conclusion not to be disputed by anyone who has seen the parents separate and afterwards side by side with the numerous distinct intermediates.

(*b*) Olearia arborescens × capillaris.

Under the designation *O. capillaris* Buch. has been grouped a series of plants differing considerably in their characters and merging eventually into *O. arborescens*, so that there is every feature of a hybrid group. Possibly the individuals with the smallest leaves and sparse-flowered small corymbs are *O. capillaris*, or it may be that the group consists of more than one jordanon. *O. arborescens* extends from about latitude 38° to Stewart Island, but *O. capillaris* is confined—so far as is known—to Mt Egmont, the Volcanic Plateau, the Northwestern Botanical District and the north of the Western Botanical District east of the Dividing Range, and generally its occurrence is quite local.

(*c*) Celmisia coriacea × Traversii (= × *C. Morrisonii* Cockayne).

For a full discussion and description of this hybrid see *Trans. N.Z.Inst.* 47, pp. 116–17, 1915. Since that time I have seen hundreds of × *C. Morrisonii* as a member of an indigenous-induced herb-field on Mount Miromiro (Northeastern Botanical District), at about 3000 ft. altitude, where the *Nothofagus* forest had been burnt. In numbers the hybrid equalled the parents. Here again the action of man has increased a hybrid population and extended its range.

5. *Hybrids between species usually growing under dissimilar ecological conditions which occasionally come together.*

This class contains 42 hybrids. The conditions under which they are produced are far from uniform. In some cases the habitats of the two parents (*e.g.* in the species of *Nothofagus*) are not very different, but on the other hand the differences may be wide and the chance of hybridisation remote (*e.g. Plagianthus betulinus* × *divaricatus*—rich alluvial soil, the one, salt swamp, the other); the hybrids may be abundant (as when the two species of *Phormium* meet), or very rare as in *Angelica decipiens* × *montana*; or, again, the certainty of what are and what are not hybrids may be masked by the presence of varietal hybrids (*e.g.* in the hybrids of the species of *Coriaria*). Many of this class of hybrids well deserve discussion, but only five cases can be dealt with here.

(a) Phormium Colensoi × tenax.

Although these species are of extremely wide range and tolerate many habitats, some suiting both, yet they seldom grow side by side. But in two localities I have seen them in close proximity along with many intermediates which were easily recognisable by the capsules, combining the erect, short, blunt form of *tenax* and the drooping long twisted form of *Colensoi*.

(b) Ranunculus Buchanani × Lyallii (= *R. Matthewsii* Cheesem.). The hybrid origin suggested in *The Vegetation of New Zealand*, p. 359.

Ranunculus Lyallii is of wide range in subalpine herb-fields of the South Island from latitude 42° 30′ and it extends to Stewart Island, but *R. Buchanani* is usually found at a higher altitude and on more stony ground; also it is confined to the Fiord Botanical District and parts of the South Otago Botanical District, adjacent thereto. In a few localities the species have come together and there are to be found many transitional forms between them, as exhibited in combinations of the huge peltate entire leaf of *Lyallii* and the smaller reniform ternatisect leaf of *Buchanani*. The large flowers also betray the *Lyallii* relationship. Cheeseman (*Manual*, p. 1133) described *R. Matthewsii* from only two specimens, which he stated came close to *R. Buchanani* but were stouter, almost glabrous (as is *Lyallii*) and with "more sparingly divided leaves and larger flowers." Specimens sent to me some years ago by Mr W. Willcox at once gave me the idea of their hybrid origin.

(c) Edwardsia microphylla × prostrata.

The first-named is a small erect tree, principally of rain-forest, its outskirts, and tussock-grassland, which has a persistent juvenile form of divaricating habit. *E. prostrata* is a prostrate shrub of exactly the same growth-form as juvenile *E. microphylla*, which grows in open situations on stony ground in a climate of but moderate rainfall. Occasionally the two species meet, as in parts of the Northeastern Botanical District, and hybrids intermediate in stature, habit, flowers and fruit appear in abundance.

(d) Olearia angustifolia × Colensoi (= *O. Traillii* T. Kirk).

Olearia Colensoi, a common shrub of subalpine-scrub in the wetter mountains of the South Island and also on the main chain of the North Island, descends to the seashore in parts of Stewart Island. There, if it meets the coastal shrub of that island, *Olearia angustifolia*, the intermediate forms known as *O. Traillii* occur, but not abundantly. The leaves are narrower than those of *O. Colensoi*, but

broader than in *O. angustifolia*, and the flower-heads show their *angustifolia* characters in their rays and purple discs. The resemblance to *O. Colensoi* is stressed in the *Manual*, p. 282. Poppelwell (*Trans. N.Z.Inst.* **48**, p. 251, 1915) suggests the hybrid origin of *O. Traillii* and describes its intermediate characters.

(*e*) Helichrysum bellidioides × H. glomeratum (= *H. Purdiei* Petrie).

These are two wide-spread species usually belonging to different associations, which, at times, grow together and the hybrid is apparently usually to be found. For many years *H. Purdiei* was considered almost the rarest species in the flora, it being known from only a few plants in one locality on the east coast of the South Island. Certainly, it looked so distinct from anything else that no one could dream it was not a valid species! But the discovery a few years ago by Mr C. E. Christensen of several distinct forms of *H. Purdiei* growing in company with *H. glomeratum* and *H. bellidioides*, not near the sea, but far inland in a mountainous part of the South Island, hinted strongly of its hybrid origin. Soon after this, but considerably further to the north, Mr B. C. Aston found *H. Purdiei* under similar conditions, while quite recently, in the neighbourhood of Lake Wakatipu, I also found the parents and the hybrid. I have also visited the habitat where Christensen made his notable discovery and found the polymorphy of the hybrid surprising, as I had only seen one individual previously in the original coastal habitat. An interesting point is that the parents belong to two distinct sections of the genus—*Xerochlæna* and *Ozothamnus*. Indeed, the latter was for many years considered a valid genus, and certainly the differences between the two species in question are far greater than between many genera. The hybrids show many combinations of the parental characters.

6. *Hybrids between species of more or less limited range which frequently grow together.*

There are only 5 hybrids in this class.

(*a*) Pittosporum pimeleoides × reflexum.

The species occur together north of latitude 35° 4′ on the floor of Kauri forest, but not everywhere. *P. pimeleoides* is a taller shrub than *P. reflexum*, with broader, more open foliage. Cheeseman writes (*Manual*, p. 60), "Both at Mongonui and Kawakawa it [*P. reflexum*] grows intermixed with the typical *pimeleoides*, together with *numerous intermediates*" (italics mine).

(b) Alseuosmia Banksii × linariifolia.

Here may also be discussed *A. Banksii × quercifolia* and *A. linarii-folia × quercifolia*, since all occur together, notwithstanding *A. querci-folia* is of fairly wide range, and its hybrids really come into class 3. *A. Banksii* and *A. linariifolia* are confined to forest in the North Auckland Botanical District; *A. quercifolia* in the southern part of its range is invariable. Carse's account of the species sufficiently proves to what an extent they cross (*Trans. N.Z.Inst.* **43**, p. 206, 1911). "There are certain forms of *Alseuosmia*," he states, "which a mere beginner can place at a glance as typical, but there are so many forms intermediate between the various species that hardly two botanists in a dozen will agree as to *which species predominates* in the particular specimen" (italics mine). Allan Cunningham described eight species, and it may well be that there are more than is usually allowed. According to the *Manual*, p. 240, "The species are exceedingly variable and difficult of discrimination."

HYBRIDS WITHIN THE SPECIES

Were knowledge sufficiently advanced, so that the true-breeding units (jordanons), which, taken together, form the ground-work of an aggregate species, could be recognised each by its special char-acters, and were a name given to each unit, then a true knowledge of these polymorphic groups (the aggregate species) might be possible, and the intervarietal hybrids be recognised. But, as in all floras, though especially in those of imperfectly botanised countries, such as New Zealand, variety after variety, for which there is no name, has its place in nature, while so many varieties as defined are them-selves aggregates, it is frequently impossible to do more than hazard a guess as to the status of any particular form. Such an one may really be a true-breeding entity, or it may be a hybrid or even an unfixed environmental form, but stable enough in that particular habitat.

There is, in fact, no definite rule according to which varieties can be taxonomically constituted any more than there is any rule governing the making of species. Further, from time to time taxo-nomic fashion changes. At present, the tendency is to "accord varieties specific rank," which action, should there be hybrids between them, would also change the status of the latter.

When a study is made of aggregate species in the field, then, so far as polymorphy goes, they fall into several classes, and there appears a distinct progression from the simple with two well-defined

varieties, by way of species with more and more varieties, up to aggregates so complex that it becomes impossible to recognise the true-breeding varieties, so great are the number of intergrading forms. In other cases the varieties may be clear-cut[1], so that intermediates when they do occur can be recognised as such, or varieties as taxonomically defined may be merely groups of related forms connected by intermediates to other so-called "varieties" of a similar character. Thus in the simpler cases where the varieties are really such (*i.e.* true-breeding entities), hybrids between them can be recognised, but, where all is confusion, their presence can only be assumed by analogy. An account of a few cases may make the foregoing remarks clearer.

Mesembryanthemum australe is an easily-recognised species which is confined to rocky stations on the coast. In certain localities two well-marked varieties occur, one with the usual pink flowers and reddish leaves, and the other with white flowers and pale-green leaves. Where there are numerous individuals, as on new stony ground, resulting from disturbance of a stony beach, there may be abundance of both varieties together with a good many forms intermediate in colour of flower and leaf. So, too, in the case of *Rumex flexuosus* there is a variety—the most common in fact—with *dark-brown* leaves. But in certain localities under the same ecological conditions along with the above there is a *green-leaved* form, and, when this happens there are also present plants intermediate in colour, which must be considered hybrids. Similarly, in the case of *Senecio bellidioides*, there are plants with tomentum and without such on the undersurface of the leaf and between these extremes are plants showing every degree in the amount of tomentum.

On the other hand there are many cases where no hybrids have been noted between varieties of similar character to the above. *Coprosma Petriei* is made up of two varieties, the one with large port-wine coloured drupes and the other with drupes faintly stained with blue, but nowhere have intermediates been seen. Neither have intermediates been noted between the var. of *Cyathodes acerosa* with

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[1] *Epilobium pedunculare* is treated in the *Manual*, p. 180, as a var. of *E. nummularifolium* of which three other vars. are recognised. But, in point of fact, as I have pointed out (*Trans. N.Z.Inst.* **50**, pp. 171–73) the latter is probably invariable, while I have shown that aggregate *E. pedunculare* is made up of a number of invariable varieties of wide range to three of which I have given varietal names. The var. *nerterioides* also is abundantly distinct from *E. nummularifolium*, but it can be taxonomically united with var. *minimum*, which also is a well-marked, certainly true-breeding group, distinguished by its very short capsule and peduncle.

red drupes and the var. with them white. On the other hand, there are drupes of many shades of blue passing into translucent white in *Coprosma brunnea*.

Agropyron scabrum is defined in the *Manual*, p. 923, as "annual or perennial, very variable." Usually it is found growing as a straggly grass with one or two long culms in the dense tussocks of *Festuca novæ-zelandiæ* or *Poa cæspitosa*, in the low tussock-grass-land. But where ground is fenced from stock for a few years this grass has assumed its true forms. These are of two classes, the one, a true tussock, and the other, of much more open and lower growth and spreading considerably by means of underground stems. But the polymorphy does not end here, for leaves varying both in breadth and hue occur on different individuals and there are also many individual differences in the length of the fruiting stems, the number and size of the spikelets and the length of the awns. There are also apparently narrow-leaved and broad-leaved races. These various characters—tussock-like, turf-like, leaves broad or narrow and of various colours, and floral distinctions—are almost certainly marks of varieties, and their combinations denote hybridism.

In some cases well-defined varieties occur which are of more or less local distribution, and at the limits of their areas, or of their particular habitat, hybridise with a more wide-spread variety. Thus *Veronica salicifolia* var. *longeracemosa*—a shrub more or less common, and perhaps the sole representative of the species in a certain part of the Egmont-Wanganui Botanical District, at the eastern boundary of its domain crosses with another var. of the same species. More striking are the many intermediate forms which occur when the swamp-dwelling *V. salicifolia* var. *paludosa* of the Western Botanical District crosses with the ubiquitous *V. salicifolia* var. *communis*.

Much more complex than the cases cited above is that of *Acæna Sanguisorbæ*. This aggregate gives rise to a remarkable variety of forms. Certain clear-cut varieties have been defined, *e.g.* *viridior*, *pilosa*, *sericei-nitens* and *minor* (this confined to the Subantarctic Islands and invariable). *Viridior* occurs side by side with the wide-spread var. *pusilla* (possibly an aggregate) and they may occasionally cross. *Pilosa* and *sericei-nitens* often occur pure in the mountains over considerable areas, but meeting var. *pusilla* much crossing takes place. The polymorphy becomes still more marked if varieties of *Acæna inermis* or *microphylla* are present, for these readily join in the crossing. Finally, if *A. novæ-zelandiæ* is present still more forms will arise.

Perhaps the maximum of multiplicity of forms is reached in the case of the shrub, or low tree, *Leptospermum scoparium*, at times also prostrate and even turf-making. Within a space of a few dozen square yards I have seen no two plants alike, and some so distinct they might pass for different species. On the other hand, in certain sphagnum bogs the individuals showed considerable uniformity, and in the North Auckland Botanical District the var. *incana* remains pure in groups throughout considerable areas. But frequently the number of forms is so great that it is impossible by means of field-observations to get any idea of the genetic constitution of the *Leptospermum* population. There are many other ultra-polymorphic species and those interested can readily find such by consulting the *Manual*.

Many of these polymorphic aggregates have greatly extended their area by the aid of man. Again Professor Henry's theory receives strong support. In the slightly wetter part of the arid North Otago Botanical District are hundreds of acres of varieties and hybrids of species of *Acæna; Leptospermum scoparium* thickets have increased to an astonishing extent since the settlement of the country; draining lowland swamps has brought into being dense crowds of polymorphic individuals of *Phormium tenax*; many forms of *Danthonia pilosa* have replaced tussock-grassland, or even artificial pasture of European grasses; the story of the incoming of *Cassinia* lowland shrubland has been already told, but in the montane and lower subalpine belts *C. Vauvilliersii, C. albida* and *C. fulvida* var. *montana* have made close thickets of an infinite variety of forms. Thus, without citing more examples it is clear that the new conditions have aided the crossing of varieties in no small degree. But what remains of primitive New Zealand can also still show much varietal hybridism, as in the following genera, to cite merely a few: *Poa, Uncinia, Nothofagus, Ranunculus, Mida* (*Santalum* of the Manual), *Colobanthus, Pimelea, Epilobium, Dracophyllum, Gaultheria, Veronica, Coprosma, Olearia, Celmisia* and *Senecio*.

There are other interesting matters connected with varietal hybridism in New Zealand, but, for the present, the above brief account must suffice. What I would particularly emphasise is, that the polymorphy of species cannot be explained by the self-evident assertion that they are "*variable*." The study of varietal hybrids is fundamental for taxonomic advance. Field observations undoubtedly have an important place in such work, but experiment must play

the final part. The New Zealand polymorphic genera—*Epilobium*, *Acæna*, *Veronica* and *Celmisia*—can supply excellent material for fairly rapid research.

LIST OF THE SUPPOSED HYBRIDS

Explanation of signs: *d* = hybrid origin more or less doubtful, or the case not sufficiently investigated; *e* = genus is endemic; *w* = one or both species not endemic, but where this sign is absent both species are endemic; *m* = that, according to *The Manual of the New Zealand Flora* intermediates occur connecting the two species; *p* = the hybrid origin had been suggested prior to this paper.

Taxaceæ

1. Podocarpus Hallii × totara.

Gramineæ

2. Hierochloe Fraseri × redolens (*w*, *m*).
3. Danthonia flavescens × Raoulii var. rubra (*m*).
4. — pilosa × semiannularis (*w*, *m*).
5. Poa anceps × pusilla var. seticulmis (*d*, *m*).
6. — Colensoi × intermedia (*m*).
7. — Kirkii × Mackayi (*m*).

Cyperaceæ

8. Scirpus aucklandicus × cernuus (*w*, *m*).
9. Uncinia cæspitosa × purpurata, filiformis or rupestris (*m*).

Liliaceæ

10. Phormium Colensoi × tenax (*w*).
11. Astelia Cockaynei × nervosa.

This name is to be substituted for *Astelia montana* (T. Kirk) Cockayne by Cheeseman in his new edition of the *Manual*, now in course of preparation, the name "*montana*" being preoccupied.

Orchidaceæ

12. Pterostylis australis × Banksii and graminea (*m*).
13. Cyrtostylis oblonga × rotundifolia (*m*).

Fagaceæ

14. Nothofagus cliffortioides × fusca (*p*).
15. — fusca × Solandri (*p*).
16. — cliffortioides × Solandri (*p*).

Moraceæ

17. Paratrophis microphylla × opaca (*m*).

Santalaceæ

18. Mida myrtifolia × salicifolia (*e*, *m*).

Polygonaceæ

19. Muehlenbeckia australis × complexa (*w*, *d*).
20. — axillaris × complexa.
21. — axillaris × ephedroides = *M. muricatella* Col.

Ranunculaceæ

22. Clematis hexasepala × indivisa (*d*).
23. Ranunculus Buchanani × Lyallii (*p*) = *R. Matthewsii* Cheesem.
24. — gracilipes × Sinclairii (*d*, *m*).
25. — depressus × rivularis (*d*).
26. — macropus × rivularis (*d*, *m*).

Magnoliaceæ

27. Wintera axillaris × colorata (*e*).

Saxifragaceæ

28. Quintinia acutifolia × serrata (*m*).

Pittosporaceæ

29. Pittosporum Colensoi × tenuifolium (*d, m*).
30. — ellipticum × tenuifolium (*d*) = *P. intermedium* T. Kirk.
31. — pimeleoides × reflexum (*m*).

Cunoniaceæ

32. Weinmannia racemosa × sylvicola (*d*).

Rosaceæ

33. Rubus australis × schmideleoides (*d*).
34. — — × parvus = *R. Barkeri* Cockayne.
35. Acæna novæ-zelandiæ × Sanguisorbæ var. pusilla.
36. — inermis × microphylla (*m*).
37. — glabra × Sanguisorbæ var. pilosa (*d*).

Leguminosæ

38. Edwardsia microphylla × prostrata.

Rulaceæ

39. Melicope simplex × ternata (*p*) = *M. Mantellii* T. Kirk.

Coriariaceæ

40. Coriaria lurida × sarmentosa (*m*).

For reason of change of name from *thymifolia* to *lurida* see W. R. B. Oliver in *Trans. N.Z.Inst.* 53, pp. 364–65, 1921.

41. Coriaria angustissima × lurida (*m*).

Elæocarpaceæ

42. Aristotelia fruticosa × racemosa, probably = *A. Colensoi* Hook. f.

Malvaceæ

43. Plagianthus betulinus × divaricatus (*d*) = *P. cymosus* T. Kirk.
44. Hoheria angustifolia × sexstylosa (*e, d*).

Violaceæ

45. Melicytus micranthus var. longiusculus × microphyllus (*d, m*).

Thymelæaceæ

46. Pimelea Gnidia × longifolia (*m*) = *P. Gnidia* var. *pulchella* Cheesem. and probably other forms.
47. — Lyallii (as in *Manual* in part, p. 614, but excluding *P. Lyallii* Hook. f. —a purely coastal species—and *P. aridula* Cockayne) × prostrata (*m*).
48. Drapetes Dieffenbachii × villosa (*d, m*).

Myrtaceæ

49. Myrtus bullata × obcordata (*p*) = *M. Ralphii* T. Kirk and × *M. Ralphii* Cockayne.

Onagraceæ

50. Epilobium Billardierianum × junceum (*w, m, p*).
51. — junceum × hirtigerum (*w, p*).

These hybrids were published by Haussknecht but there is doubt as to what he means by *E. junceum*.

52. Epilobium Billardierianum × pubens (*w, p*).
53. — glabellum × pubens (*w, d*).
54. Fuchsia Colensoi × excorticata (*m*).

Araliaceæ

55. Nothopanax anomalum × simplex (*d*) = *N. parvum* (T. Kirk) Cockayne.

Umbelliferæ

56. Apium filiforme × prostratum (*w, m*).
57. Anisotome Haastii × pilifera (*e*) = *A. pilifera* (Hook. f.) Cockayne and Laing var. *pinnatifida* T. Kirk.
58. Angelica decipiens × montana.

Cornaceæ

59. Corokia buddleioides × Cotoneaster (*e, p*) = *C. Cheesemanii* Carse.

Ericaceæ

60. Gaultheria antipoda var. microphylla × perplexa (*d, m*).
61. — oppositifolia × rupestris = *G. fagifolia* Hook. f.

Epacridaceæ

62. Dracophyllum longifolium × montanum (*m*).
63. — Lessonianum × longifolium (*m*).

Gentianaceæ

64. Gentiana bellidifolia × patula (*d, m*).

Scrophulariaceæ

65. Veronica macroura × salicifolia. (*m*).
66. — leiophylla × salicifolia var. communis = *V. Kirkii* J. B. Armstg. and other forms.
67. — macrocarpa × salicifolia (*m*) = *V. macrocarpa* Vahl var. *affinis* Cheesem. and other forms.
68. — elliptica × salicifolia var. communis (*w*) = *V. Lewisii* J. B. Armstg.
69. — — × salicifolia var. (*w, d*) = *V. amabilis* Cheesem. var. *blanda* Cheesem.
70. — angustifolia × salicifolia var. Atkinsonii = × *V. Simmonsii* Cockayne.
71. monticola × Traversii (*d*).
72. glaucophylla × Traversii (*d*).
73. — lævis × salicifolia var.
74. — × tetragona.
75. — Buchanani × pinguifolia (*m*) = *V. Buchanani* Hook. f. var. *major* Cheesem. and other forms.
76. — buxifolia (probably) × lycopodioides (or some other whipcord veronica) × *V. cassinioides* Petrie.
77. — epacridea × Haastii (*d, m*).
78. — Lyallii × one of the whipcord veronicas × *V. loganioides* J. B. Armstg.
79. — diffusa × lanceolata (*m*).
80. — catarractæ var. × Lyallii (*d*).

Rubiaceæ

81. Coprosma propinqua × robusta (*m*) = *C. Cunninghamii* Hook. f.
82. — Banksii × Colensoi = *C. Astoni* Petrie and many other forms.
83. — cuneata × depressa (*d*).

Caprifoliaceæ

84. Alseuosmia linariifolia × quercifolia (*e, m*).
85. — Banksii × quercifolia (*e, m*).
86. — — × linariifolia (*e, m*).

Compositæ

87. Lagenophora petiolata × pumila (*d, m*).
88. Brachycome pinnata × Sinclairii (*d, m*).
89. Olearia angustifolia × Colensoi = *O. Traillii* T. Kirk.
90. — arborescens × capillaris.

Compositæ—*continued*

91. Olearia ilicifolia × macrodonta (*d, m*).
92. — — × moschata.
93. — — × sp. of *Olearia* = *O. mollis* (T. Kirk) Cockayne.
94. — excorticata × ilicifolia.
95. — Haastii × moschata.
96. — — × oleifolia (*m*).
97. — cymbifolia × nummularifolia.
98. — avicenniæfolia × odorata = *O. Willcoxii* Petrie.
99. — coriacea × Forsteri.
100. Celmisia discolor, or perhaps brevifolia × Walkeri (*m*).
101. — — × Sinclairii (*m*).
102. — coriacea × Lyallii.
103. — — × spectabilis = *C. Boweana* Petrie and other forms.
104. — — × Traversii (*p*) = *C. Morrisonii* Cockayne.
105. — — × verbascifolia.
106. — — × gracilenta.
107. — — × sp. of Celmisia = *C. flaccifa* Cockayne.
108. — spectabilis × Traversii (*p*) = × *C. Christensenii* Cockayne.
109. — — × petiolata (*p*) = *C. mollis* Cockayne.
110. — petiolata × verbascifolia = *C. lanigera* Petrie and other forms.
111. — Lyallii × spectabilis (*p*) = *C. pseudo-Lyallii* (Cheesem.) Cockayne.
112. Gnaphalium Keriense × Lyallii (*m*).
113. — trinerve × Helichrysum bellidioides (*d*).
114. — Mackayi × Traversii (*m*).
115. Raoulia australis × apice-nigra.
116. — — × lutescens (*d*).
117. Helichrysum bellidioides × prostratum (*n*).
118. — — × glomeratum (*p*) = *H. Purdiei* Petrie.
119. — — × Sinclairii (*p*) = *H. Foverakeri* Cockayne.
120. — coralloides × Selago (*d*).
121. Cassinia fulvida × leptophylla.
122. — — var. montana × Vauvilliersii.
123. — albida × Vauvilliersii.
124. — — × fulvida var. montana.
125. Cotula lanata × plumosa (*w, d*) = *C. propinqua* Hook. f.
126. — pectinata × sericea.
127. — dioica × pulchella (*m*).
128. Senecio Lyallii × scorzoneroides.

Addenda (but not included in total given in body of paper).

129. Acæna inermis × Sanguisorbæ (one or more of its vars. (*w*)).
130. — microphylla × Sanguisorbæ (one or more of its vars. (*w*)).

PERMEABILITY

By WALTER STILES

CHAPTER XII (*continued*)

QUANTITATIVE RELATIONS IN THE PENETRATION OF
DISSOLVED SUBSTANCES INTO PLANT CELLS (*continued*)

Influence of temperature on the rate of absorption. The effect of
temperature on the rate of absorption was examined by Stiles and
Jørgensen (1915 *b*) for the case of the absorption of hydrochloric acid,
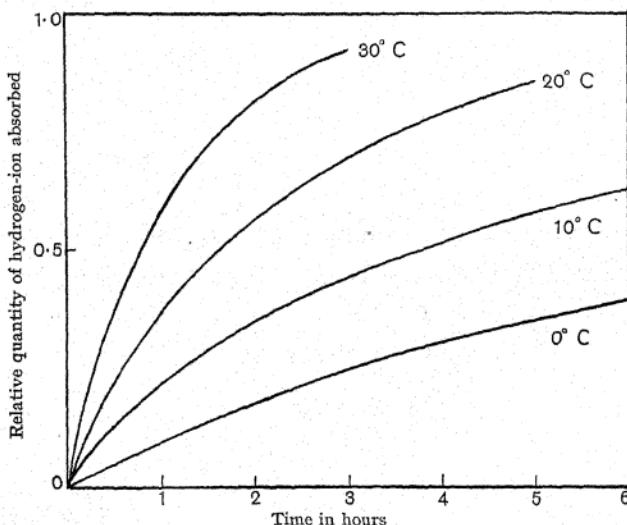
Fig. 15. Curves to illustrate the absorption of hydrogen-ion by potato tuber
tissue immersed in 0·0011 *N* hydrochloric acid at various temperatures.
(From the data of Stiles and Jørgensen.)

or rather the hydrogen ions of that acid, by potato tuber. The tissue
was used in the form of circular disks 1·0 cm. in diameter and 0·2 cm.
in thickness, 20 disks being immersed in 100 c.c. of the acid solution.

The latter was used in a concentration of 0·0011 N and changes in its value with time were determined electrometrically by means of the hydrogen electrode. The absorption of the acid was followed in this way at temperatures of 0°, 10°, 20° and 30° C. The relation between time and absorption found in these experiments is represented graphically in Fig. 15. The relation is almost a logarithmic one, so that the equation representing the rate of absorption is approximately

$$\frac{dx}{dt} = k (A - x),$$

where $\frac{dx}{dt}$ is the rate of absorption at any time when x is the diminution in the concentration of acid in the external solution and A represents the initial concentration. In this equation k is determined by the temperature and in Table XLIV are shown values of k calculated for the different temperatures employed. From these results it appears that the rate of absorption of hydrogen ions by potato tuber is increased about 2·2 times for every rise of temperature of 10° C. within the temperature range 0° C. to 30° C.

TABLE XLIV

Influence of Temperature on the Rate of Absorption of Hydrogen Ions by Potato Tuber. (Data from Stiles and Jørgensen)

Temperature in centigrade degrees	"k"
0	0·036
10	0·081
20	0·174
30	0·380

This temperature coefficient (Q_{10}) is that characteristic of chemical reactions rather than of physical reactions such as diffusion or adsorption. But having regard to the great variations in the value of the temperature coefficient in different cases of the absorption of water by plant tissue, it would clearly be unwise to draw far-reaching conclusions with regard to the nature of the process of absorption of hydrogen ions from the temperature coefficient alone. But from the magnitude of the absorption it seems clear that something more than simple diffusion alone is required to account for the process. Thus, after three hours in the acid at 30° C. the potato tissue had absorbed so much acid that the absorption ratio was 39 and there was no indication that equilibrium had been reached.

Whether chemical action or absorption is mainly responsible for this result cannot at present be said definitely any more than it can be for the case of salts.

A few observations are on record indicating that the rate of intake of dyes is increased by rise in temperature. Pfeffer (1886) found this the case with methylene blue and Endler (1912 b) for neutral red. Collander (1921) has recently made some observations on the influence of temperature on the intake of some sulphonic acid dyes (orange G and cyanol), and concludes that temperature has a considerable effect on the intake of these dyes by the pith cells of *Tropæolum*. Similar results were obtained with the parenchyma cells of perianth leaves of *Hyacinthus*, although the temperature effect was not so great. As, however, the intake of dye was not followed with time, information relative to the temperature coefficient is not forthcoming.

Influence of light on the rate of absorption. The influence of light on the rate of absorption of sodium chloride by palisade cells of *Acer platanoides*, *Salix babylonica* and *Buxus sempervirens* was examined by Tröndle (1918 b) by the deplasmolytic method. The results indicate that increasing illumination increases the rate at which the salt passes into the cells up to a maximum with a certain light intensity. This intensity varies with the different cells examined; with *Acer platanoides* it was about 1250 metre candles, with *Salix babylonica* about 9375 metre candles and with *Buxus sempervirens* about 1500 metre candles. Results obtained previously by the same author (1910) by the method of permeability coefficients gave similar indications. On the other hand, Ruhland (1911), using this latter method, found that light was without measurable influence on the permeability of the cells of the sugar beet leaf to sucrose, glucose and fructose.

Using this same method Lepeschkin (1909 b) showed that the cells of the pulvini were more rapidly penetrated by dissolved substances after illumination than in the dark, an observation confirmed by Blackman and Paine (1918) by determining the exosmosis of electrolytes by means of changes in the electrical conductivity of the external liquid. For reasons already given, neither the method of permeability coefficients nor the deplasmolytic method can be regarded as capable of yielding exact quantitative data with regard to the passage of substances into the cell. However, a more rapid rate of deplasmolysis can probably be accepted as indicating more rapid entrance of dissolved substance, and a higher permeability coefficient as indicating the same thing.

Kahho (1921 *b*) found that upper epidermal cells of the leaf of the red cabbage placed in a 0·48 *N* solution of sodium iodide at a temperature of 23–24° C. were killed more rapidly in light than in the dark. Similar results were obtained with sodium bromide and sodium thiocyanate. These results were attributed by Kahho to a more rapid entrance of the salts in question in the light than in the dark.

Influence of the thickness of the tissue on the rate of absorption. Höfler and Stiegler (1921), using the plasmometric method, observed that urea entered the epidermal cells of the stem of *Gentiana Sturmiana* much more slowly in very thin sections which contained only the epidermal layer, but this uninjured, than in thick sections. The explanation of this is not forthcoming; it might, perhaps, be correlated with the observations of Tröndle (1921) on the effect of wounding on permeability to which reference is made in the next chapter.

On the course of absorption in general. The curves obtained by Stiles and Kidd for the course of absorption of salts show that there is a rapid intake of salt for the first 15 or 30 minutes after which absorption follows an approximately logarithmic course towards the position of equilibrium. How exactly the latter part of the curve, or the whole of it, is represented by a logarithmic equation, cannot be said; in the case of the absorption of hydrogen ions by potato tuber the approximation was near enough for the assumption of this relation in the calculation of the influence of temperature.

As the position of equilibrium differs in the case of every substance and of every concentration of the same substance, at any rate as far as existing data indicate, it is perfectly clear that the rate of absorption cannot be regarded as a measure of the permeability of cell membranes to the substance. For if the permeability of the cell membranes to potassium chloride and calcium chloride were the same, the rate at which the calcium salt entered the cell would soon slow down as compared with the rate at which the potassium salt entered, on account of the different position of the equilibrium.

The explanation given by Fitting (1915) of the slowing down of the rate of deplasmolysis is that the salt itself lowers the permeability of the cell to the salt. That this explanation is inadequate becomes clear from the fact that the position of equilibrium bears a definite relation to the concentration of the external solution, and that in low concentrations the absorption ratio may be many times unity, a result which cannot be explained on the ground of changes in permeability of the membrane alone. As the concentrations used by

Fitting are very high, it is to be expected that the position of equilibrium will be attained when the absorption ratio has reached a position considerably below unity.

Tröndle considered his results as indicating that there was a rapid intake of salt during the first 10 minutes of experiment, during which time the intake of salt was proportional to the time of action and independent of the concentration, after which the rate of intake gradually lessened according to a logarithmic relation. That Tröndle's method is capable of giving such accurate data I very much doubt for reasons already stated. Tröndle's theoretical conclusions from these results are somewhat surprising; they will be mentioned on a later page.

Höfler (1918 b, 1919) investigated the absorption of potassium nitrate by cells of *Tradescantia elongata* and *Rhœo discolor* by means of the plasmometric method described in the last chapter. This method, it will be recalled, attempts to measure the intake of salts by individual cells. Höfler found extraordinarily wide variations in the rate of absorption of the salt by different cells of the same tissue. Thus in a section of parenchymatous tissue of *Tradescantia elongata* the quantity of salt absorbed by individual cells in the same time varied between 0·009 gm. mol. and 0·043 gm. mol. with a mean value of 0·022 gm. mol. Moreover, the quantity of salt apparently absorbed by the same cell may undergo as wide variations with time. These changes in rate of intake appear quite independent of the abnormally high rate of intake which is an indication of the death of the cell. A general decrease in the rate of intake as observed by Fitting was not observed by Höfler until the lapse of about a day or longer from the first immersion of the tissue in the solution.

The course of absorption of the two ions of a number of salts by whole plants of a considerable number of different species was investigated by Pantanelli (1918) by analysing the external solutions for both ions. He came to the conclusion that the two ions are absorbed independently throughout the whole course of absorption. The rate of absorption varied greatly with different ions and different salts, and ions that were absorbed most rapidly by one species were not necessarily absorbed with any great rapidity by another species. Generally speaking, other factors being equal, unicellular organisms or those rich in protoplasm, absorb ions much more rapidly than multicellular organisms or those poor in protoplasm.

Pantanelli found that in some cases oscillations occurred in the total quantity of ion taken in by certain of the plants he examined,

indicating alternating periods of absorption and excretion. This was observed for example in *Vicia Faba* in potassium bromide, sodium monohydrogen phosphate, calcium nitrate and barium nitrate; *Valonia utricularis* in ammonium sulphate, magnesium sulphate, potassium dihydrogen phosphate, ammonium nitrate or potassium bromide; yeast in zinc sulphate or calcium chloride. But as the method of Pantanelli involves the use of different plant material for each analysis, it seems possible that the differences recorded resulted from differences in the absorbing capacity of the various batches of plants used. Pantanelli is aware of this difficulty, but thinks that as a source of error it is negligible because he had four plants in every vessel and took care to use plants having equal development both of root and shoot.

I think it is likely Pantanelli has underrated the magnitude of variation possible among plants that appear very similar to the eye. Miss Redfern (1922 a) performed similar experiments on the absorption of calcium and chlorine ions from solutions of calcium chloride by living plants of *Pisum sativum* and *Zea Mays*. Careful selection of plants that appeared equally developed was made at the commencement of the experiment, each plant was grown singly in water culture and for any determination of absorption separate analyses were made of the solutions in which six plants had been growing. From these results it was possible to calculate the probable error of the determination of absorption, and although owing to the small number of plants no great exactitude could be claimed for the results, yet these brought out very plainly the great variations that may occur in absorption by apparently exactly similar plants. The results also showed that within the limits of probable error there was no indication of such a periodicity in absorption as that recorded in a number of cases by Pantanelli, absorption proceeding continuously to an equilibrium value attained in the case of peas after about 24 hours, and in the case of maize after about 48 hours. Subsequent excretion of the salt appears to be due to the approach of death of the plant. Further work with other plants is necessary, however, before it can be denied with certainty that the apparent oscillations observed by Pantanelli can be definitely explained as due to differences between individual plants.

THE INFLUENCE OF THE PRESENCE OF ONE DISSOLVED
SUBSTANCE ON THE ABSORPTION OF ANOTHER

So far, our consideration of the intake of dissolved substances has been limited to substances presented to the plant in pure solution. We have now to consider how this intake is affected when more than one substance is present in solution in the external medium. It has already been mentioned in Chapter IV that the presence of a non-electrolyte like sugar or glycerol reduces the rate of diffusion of an electrolyte, while in Chapter V reference has been made to the retardation or inhibition of diffusion through porcelain membranes that can be brought about by blocking the pores of the membrane with some substance. It would not then be surprising to find that the presence of one substance in the external solution should hinder the intake of another.

That the presence of a second substance dissolved in the external medium reduces the harmful action of a toxic substance in the solution external to living tissue has long been recognised (cf. Brenchley, 1914 b, Stiles and Jørgensen, 1914 b). Such a result does not, in itself, prove that the actual entrance of the toxic substance is prevented or retarded. The seat of the depoisoning action may be inside the plant. However, there is considerable evidence that this "antagonism" between dissolved substances is actually due to a mutual hindrance to their entrance into plant cells; this evidence it will be necessary for us to discuss.

This antagonistic action was brought into prominence by the work of Loeb (1900, 1901, 1902, 1905, 1906; Loeb and Gies, 1902) on the development of the eggs of the marine fish *Fundulus*. Development of these eggs is inhibited if they are put immediately after fertilisation into a pure solution of sodium chloride having the same concentration as that of this salt in sea water. But if a small quantity of the salt of a bivalent metal such as calcium, strontium, magnesium, or even lead, is added to the sodium chloride solution, development of the egg into an embryo is able to proceed. The result depends on the kations, as different salts of the same metals are able to act equally well as depoisoners. The antagonism between monovalent and bivalent kations is also shown in the depoisoning action of a monovalent kation towards the poisonous action of a bivalent metal such as zinc. Slight antagonism was also observed between two divalent kations, as, for example, strontium and magnesium.

With regard to quantitative relations between the two ions, the quantity of the depoisoning ion required to inhibit the action of the poisonous ion varies with the concentration of the latter. Thus a concentration of 0·25 N NaCl is harmless to *Fundulus* eggs; with a concentration of 0·625 N NaCl one bivalent ion is required to render 1000 sodium ions harmless. Between these two concentrations the number of bivalent ions relative to the number of sodium ions is less the lower the concentration of the sodium chloride, while above a certain concentration of sodium chloride it is impossible to inhibit the toxic action of the sodium chloride.

These antagonistic effects only appear so long as the fish is surrounded by the egg membrane. Loeb concludes that the membrane is the seat of the antagonistic action, and that it is here that the ions mutually hinder one another in penetrating into the egg.

Loeb's work on antagonism in animals was followed up in plants by Osterhout, who devised a number of methods of attacking this question. In his earliest experiments antagonism in plants was examined by the reduction of toxic action in mixed solutions as compared with the toxicity of pure solutions containing the same substances in the same concentration. The first experiments were with marine plants (Osterhout, 1906 *a, b, c*). It was found that while some marine plants are quickly killed when placed in distilled water, others can live a long time in this medium[1]. The latter plants are, however, killed much more quickly when placed in a pure solution of sodium chloride isotonic with sea water. The toxic action of the sodium chloride is removed to a very great extent by the addition of a little calcium chloride, for in a mixture containing 1 c.c. of 3/8 M calcium chloride + 100 c.c. 3/8 M sodium chloride, the plants live nearly as long as in distilled water. If some potassium chloride is added to the mixture the plants live longer than they do in distilled water, while with further addition of magnesium chloride and magnesium sulphate, the plants live practically as long as in sea water.

The toxicity of sodium chloride is also reduced by the addition of potassium chloride or magnesium chloride, but not so much as by the addition of calcium chloride. The addition of potassium chloride + calcium chloride reduces toxicity more than magnesium chloride + calcium chloride, and this mixture more than magnesium chloride + potassium chloride.

[1] Possibly owing to exosmosis being less in the case of the latter (cf. Osterhout, 1917 *b*).

Similar results were obtained by Osterhout (1907) with a number of freshwater and terrestrial species including *Spirogyra*, *Vaucheria*, *Lunularia* gemmæ and *Equisetum* spores, as well as wheat seedlings and other flowering plants. In this and subsequent investigations, Osterhout (1907, 1908 c) introduced a second method of investigating antagonism, namely, by determination of differences in growth rate. Wheat seedlings, for example, were grown with their roots in the experimental solutions, and the amount of growth determined by measuring the increase in length of the roots in the various solutions after a definite time. Various chlorides and nitrates were employed. In this way it was shown that root development proceeded more rapidly in solutions containing both salts of sodium and potassium, sodium and ammonium, sodium and calcium, and sodium and magnesium, than in pure solutions of the same concentration (0·12 *M*). For each pair of salts there is a definite ratio of the two constituents in which the rate of growth is a maximum.

In a separate investigation (1908 *a*) Osterhout showed that the toxicity of magnesium salts (chloride, sulphate and nitrate) was reduced by the addition of the corresponding potassium salt. Not only is the toxicity of the magnesium reduced by the potassium, but the toxicity of potassium is reduced by the presence of a magnesium salt, for a solution containing both the potassium and magnesium salt is less toxic than a pure solution of either salt in the same concentration.

Much the same method was used by Hansteen (1910) who employed a Norwegian variety of wheat, seedlings of which were maintained for 14 days with their roots in the experimental solutions and the root development then estimated by determining the dry weights of the roots. Nitrates were used in concentrations considerably lower than those employed by Osterhout. By this method a definite antagonism was observed between potassium and calcium, a rather slight antagonism between potassium and magnesium, and a very little, scarcely noticeable, antagonism between potassium and sodium. Antagonism was observed in solutions having a concentration as low as 0·00125 *N*.

Further work on these lines was carried out by McCool (1913) who worked with wheat and Canadian field peas allowed to grow in the experimental solutions for 21 to 30 days. Growth was estimated by measurement of length and determination of dry weights of roots and shoots. Antagonism between calcium and the following metallic ions was found: magnesium, potassium, sodium, ammonium, barium

and strontium. Antagonism was also found between barium and potassium, barium and magnesium, and between strontium and the following: potassium, sodium and magnesium. McCool also observed antagonism between sodium and potassium and between sodium and ammonium. The poisonous action of manganese is reduced by the presence of calcium, potassium, sodium or magnesium.

Antagonism can also be demonstrated by means of determinations of the electrical conductivity of living tissue. It has been mentioned earlier that dead tissue possesses a much greater electrical conductivity than living tissue. Osterhout (1912 *a*, 1914 *f*, 1915 *b*) showed that tissue of *Laminaria* lost in electrical resistance much more rapidly in a solution of sodium chloride having the same conductivity as sea water than in a solution of pure calcium chloride of the same conductivity, while in a solution of this same conductivity containing sodium chloride and calcium chloride in the proportion of 1000 molecules of the former to 15 of the latter, *Laminaria* maintained its original resistance for 24 hours, indicating that in the mixed solution the cells of the alga remain alive considerably longer than in pure solutions of the same conductivity. By this method antagonism has also been demonstrated between sodium chloride and magnesium chloride, sodium chloride and hydrogen chloride (Osterhout, 1914 *i*, 1915 *a*), sodium chloride and sodium taurocholate (Osterhout, 1919 *b*) and between sodium chloride and a purine, caffeine, and an alkaloid, cevadine sulphate (Osterhout, 1919 *d*). Antagonism between sodium acetate and sodium sulphate, and also between sodium citrate and a number of other sodium salts (chloride, sulphate, nitrate, iodide and thiocyanate) has been shown by Raber (1917, 1920 *b*) by the same method.

Brenner (1920) considers that he has demonstrated the existence of antagonism between hydrochloric acid and a number of salts by the following experiments. He had earlier (1918) examined the resistance of hypodermal cells of red cabbage to acids and alkalies in low concentrations, using plasmolysis and deplasmolysis as tests of the vitality of the cells examined. Pieces of tissue were placed in a solution of 20 per cent. sucrose containing the acid in definite concentration and left in it for the experimental time (ten minutes to four hours). The pieces of tissue were then transferred to 10 per cent. sucrose, then into 5 per cent. sucrose for 30 minutes and, finally, into distilled water for the same length of time. If the cells deplasmolysed normally they were considered to be living. If dead, the protoplast was either disorganised or was in a fixed plasmolysed

condition. In this way the critical concentration of a number of acids, that is, the concentration necessary to injure the cells in a certain time, was found for different plant cells. The critical concentration of hydrochloric acid, for example, in the case of hypodermal cells of red cabbage immersed for four hours in the sucrose solution containing the acid, was $N/700$, corresponding to a hydrogen-ion concentration of $1·4 \times 10^{-3}$.

In his experiments on antagonism a similar procedure was employed, the hydrochloric acid in various concentrations being added to a number of salts in solutions which gave approximately equal plasmolysis. The sections were placed for 20 minutes in these plasmolysing solutions, then for four hours in the solution of salt + acid. They were then transferred successively into a half-strength salt solution, a quarter-strength salt solution and distilled water. The concentrations of salt used, the concentration of hydrochloric acid in the salt solution, and the actual hydrogen-ion concentration, determined by the hydrogen electrode, of the mixed solution, are shown in Table XLV.

TABLE XLV

Critical Concentration of hydrochloric acid in regard to hypodermal cells of red cabbage in presence of various salts and sugars.

(Data from Brenner)

Salt or sugar	Concentration of salt or sugar in per cent.	Critical concentration of hydrochloric acid	Actual hydrogen-ion concentration of the solution
Sodium chloride	2·2	1×10^{-3}	$8·91 \times 10^{-4}$
Potassium nitrate	3·75	$1·25 \times 10^{-3}$	$1·29 \times 10^{-3}$
,, chloride	2·8	$1·67 \times 10^{-3}$	$1·38 \times 10^{-3}$
,, sulphate	5·0	$2·5 \times 10^{-3}$	$4·68 \times 10^{-4}$
Magnesium nitrate	8·8	1×10^{-3}	$1·09 \times 10^{-3}$
,, chloride	7·0	$2·5 \times 10^{-3}$	$3·16 \times 10^{-3}$
,, sulphate	16·1	4×10^{-3}	$1·12 \times 10^{-3}$
Calcium nitrate	6·5	2×10^{-3}	$1·95 \times 10^{-3}$
,, chloride	6·2	4×10^{-3}	$5·50 \times 10^{-3}$
Glucose	—	$1·43 \times 10^{-3}$	$8·90 \times 10^{-4}$
Sucrose	20	$1·43 \times 10^{-3}$	$8·71 \times 10^{-4}$

It will be observed from the data presented in this table that the different substances examined affect very differently the resistance of the cells to hydrochloric acid. Sodium chloride has the same effect as sugar, while potassium sulphate lessens the resistance of the cells to about half. All the other salts, on the other hand, raise considerably the resistance of the cells to acids, as a much higher hydrogen ion concentration is necessary to injure the cells in the presence of the salts.

From these and other observations, as, for example, on the inhibition of the toxic action of salts of copper and heavy metals by salts of the alkali and alkaline earth metals in regard to the germination of fungus spores (J. F. Clark, 1901, 1902; Hawkins, 1913) there can be no doubt that antagonism is a very general phenomenon. But from these investigations, in which antagonism is determined by time taken to kill, amount of growth, or germinative capacity, there is no direct evidence that the antagonistic action of the substances concerned is due to a mutual hindrance to the absorption of the two substances. The antagonism may result from reactions in the external solution, or in the cells themselves after the entrance of the substances. It is clear that the former can scarcely be a general explanation, as antagonism has been observed to take place between so many substances which do not react with one another. Evidence that the antagonism is indeed due, at any rate in some cases, to a mutual hindrance to the entrance of substances into the cell, has been forthcoming from other lines of experimentation.

An observation of Benecke (1907), which appears to have been somewhat overlooked, affords more direct evidence of the effect of one electrolyte on hindering the entrance of another. As is well known, the entrance of an iron salt into the cells of a species of *Spirogyra* containing tannin, is rendered evident by the formation of a green or blue compound. Benecke observed that the entrance of ferrous sulphate into such cells is much delayed by the addition of a calcium salt to the solution of ferrous sulphate. This observation was confirmed by Szücs (1910), who also found that the same result could be brought about by a number of salts, the antagonising action being a function of the valency of the kation, the higher the valency the greater the antagonising action.

These results appear, at first sight, to be in complete discordance with those of Miss Williams (1918 *b*) who found that cells which did not normally absorb ferric chloride would do so after treatment with solutions of various nitrates. It will be observed, however, that in Benecke's experiment the two antagonising salts were present together in the same solution, whereas in Miss Williams' experiments they were present singly and presented to the tissue one after the other.

The non-entrance of the toxic substance in the reduction of toxic action by addition of some other substance to the toxic solution is also indicated by observations on the effect of barium salts on cells of *Spirogyra*. Osterhout (1916 *e*) showed that the chloroplasts of this

alga underwent a peculiar and characteristic contraction in solutions of barium chloride as dilute as 0·0001 M. Strontium chloride produced the same contraction in concentrations of 0·001 M and upwards. Chien (1917) found the same contraction could be produced with cerium chloride in a concentration of 0·00005 M, in a large species of *Spirogyra*, but not in a small one. In the smaller species it was found that the effect of barium chloride was inhibited by addition of calcium chloride or cerium chloride to the solution in the correct proportions, but that no antagonism could be observed between barium and strontium.

A plasmolytic method has been used by Osterhout (1911, 1912 c, 1913 b). It was found that cells of a species of *Spirogyra* were just plasmolysed in 0·2 M calcium chloride and in 0·38 M sodium chloride, but were not plasmolysed in 0·195 M calcium chloride nor in 0·375 M sodium chloride. However, the solution obtained on mixing 10 volumes of the 0·375 M sodium chloride solution with 1 volume of the 0·195 M calcium chloride solution at once brought about plasmolysis. The failure of the solutions of the single salts in these concentrations to bring about plasmolysis is held to indicate the penetration of the solute into the cells in these cases, while the occurrence of plasmolysis with the mixed solution indicates the non-entrance of the solutes from the mixed solution. As the osmotic concentration of the mixed solution is so little different from (actually a little less than) that of the pure sodium chloride solution, and as the concentration of sodium chloride is reduced by only about 9 per cent., it seems improbable that the smaller rate of entrance of the salt on account of the lower concentration of sodium chloride would alone be sufficient to account for plasmolysis taking place in the mixed solution, and it seems most likely that the capacity for the molecules of sodium chloride to enter the cell is reduced.

The influence of one salt on the intake of another by young roots of yellow lupin has been investigated by Kahho (1921 d) by the tissue extension method. It will be recalled that Kahho found kations absorbed in the order K, Na, Li, Mg, [Ba, Ca], while anions fall into the series [Br, I, NO₃], Cl, tartrate, SO₄, citrate. When the entrance of single salts and that of mixtures of two salts are compared, the solutions in all cases being isotonic, it is found that the entrance of any kation in the series is retarded by the presence of any other kation to the right of it in the series, the further the kation lies to the right the greater the retarding effect. Thus, potassium chloride enters much more rapidly than lithium chloride presented in the

same osmotic concentration, but from a mixture of the two having the same total osmotic concentration ($0 \cdot 15$ KCl + $0 \cdot 048$ M LiCl), salt enters a little faster than from the pure lithium chloride solution ($0 \cdot 192$ M) but much slower than from the pure potassium chloride solution ($0 \cdot 200$ M).

The same rule was found to hold for anions. The action of any salt in furthering or retarding intake was found to be the sum of the action of its two ions.

While it is clear from earlier considerations put forward in this chapter dealing with the unequal absorption of ions, that the plasmolytic test is not free from objection, direct evidence that antagonistic action is due to mutual hindrance to intake is forthcoming from chemical analysis of tissues and colorimetric determinations in cells and external solutions. By such means it has been shown by Szücs (1911, 1912) that roots of *Cucurbita Pepo* rapidly absorb enough copper from pure solutions of copper sulphate not only to inhibit the response of the hypocotyl and root to gravity and light, but also to give a very definite qualitative test for copper. If aluminium chloride is added to the solution of copper sulphate, the inhibition of the geotropic and phototropic reaction is delayed and the copper is absorbed to a much less extent in the same time. The maximum depoisoning effect is produced with a ratio of $0 \cdot 15$ N aluminium chloride to $0 \cdot 025$ N copper sulphate.

From water culture experiments with *Eriophorum vaginatum*, *Phragmites communis* and *Carex riparia*, Stoklasa, Šebor, Týmich and Cwacha (1922) conclude that they find antagonism between aluminium and ferric ions. These plants were maintained with their roots in complete nutrient solutions which in some cases contained in addition aluminium nitrate, in others ferric nitrate and in yet others both these salts. By analysis of the experimental solutions after the lapse of 13 days it was found that less iron and aluminium were absorbed from the solution containing both these salts than from those containing only one, although the conditions were otherwise equal and the concentration of both the aluminium nitrate and the ferric nitrate was the same, namely $0 \cdot 002$ N, as in the solutions containing only one of these salts. Thus, in the experiments with *Phragmites communis* the quantity of aluminium absorbed from the solution containing this without iron was $51 \cdot 3$ mgm., the quantity of iron absorbed from the solution containing this without aluminium was $46 \cdot 6$ mgm. while from the solution containing both, $27 \cdot 3$ mgm. aluminium and $8 \cdot 4$ mgm. iron were absorbed.

By direct tests of the cell sap of *Nitella*, Miss Brooks (1922) has shown that lithium, calcium and strontium ions enter the cells of this plant less rapidly from mixed balanced solutions than when presented singly. From determinations of the electrical conductivity of the sap of the same species Osterhout (1922 *b*) concludes that the nitrate ion rapidly penetrates the cells when sodium nitrate is presented to the plant in pure solution, whereas when the sodium nitrate is balanced by the addition of calcium nitrate the rate of entrance is much slower.

So far we have considered only the antagonism between inorganic salts, or, more correctly, between ions, for, as we have already seen, the ions of a salt may be absorbed to different extents. Antagonism has also been observed between inorganic salts and organic substances such as dyes and alkaloids. Thus, von Eisler and von Portheim (1909) found the toxic action of quinine reduced by the presence of salts of sodium, calcium, magnesium and aluminium.

Szücs (1912) examined particularly the antagonism between quinine hydrochloric and potassium nitrate, calcium nitrate and aluminium nitrate in the case of *Spirogyra*. The absorption of the quinine is made visible by the precipitation of tannin in the cells, while as a test of vitality, centrifuging the filaments was employed, the plasma and chloroplasts remaining fixed if the cells are dead, but not otherwise. Each of the three salts used was able to antagonise the toxicity and entrance of the quinine, the depoisoning effect increasing with the valency.

That antagonism is actually due to the non-entrance of the toxic substances is indicated by the observation of Szücs on the toxic action of piperidine in presence and absence of various inorganic salts. For some unknown reason the absorption of piperidine is not hindered by inorganic salts, and the toxicity of the piperidine is increased rather than diminished by the addition of an electrolyte.

Following up the work of Szücs on the antagonistic action of aluminium nitrate and quinine hydrochloride, Weevers (1914) examined the depoisoning action of aluminium chloride towards a number of organic substances. The tissue used was red beet parenchyma, the exosmosis of the red pigment from the cells being taken as a criterion of toxic action. By this method it was shown that aluminium chloride could antagonise a number of organic poisons, namely, quinine hydrochloride, chloral hydrate, formaldehyde, amyl alcohol, ethyl alcohol, ether and chloroform. Antagonism was also observed between all these substances, except formaldehyde and ethyl

alcohol, on the one hand, and zinc sulphate, cobalt chloride and manganese acetate on the other. Antagonism was also observed between quinine hydrochloride and copper acetate, and between chloral hydrate and copper sulphate. Little or no antagonism was found between these compounds and sodium and potassium salts, nor between formaldehyde and any salt except aluminium chloride. With each organic substance the depoisoning action of the trivalent aluminium ion was found to be greater than that of the divalent ions examined.

The antagonism between an aniline dye and an electrolyte was also demonstrated by Szücs (1910). The dye selected for most of Szücs' experiments was methyl violet, the intake of which by *Spirogyra* cells was examined by determining the time required for the cells to acquire a standard depth of colour. When, for example, potassium nitrate was present in a variety of concentrations (0·001 N to 0·08 N) in 0·0001 per cent. methyl violet solution, the time taken for the standard colouration of the cells to be acquired was found to vary with the concentration of the salt. The same was the case when calcium nitrate or aluminium nitrate was employed. In Table XLVI are exhibited the results obtained.

TABLE XLVI

Time taken for cells of *Spirogyra* to reach a definite intensity of colour when immersed in methyl violet solution containing various quantities of electrolyte. (Data from Szücs)

Concentration of methyl violet in per cent.	Electrolyte	Concentration of electrolyte in normalities	Time in minutes required for the acquisition of standard tint
0·0001	Potassium nitrate	—	2·0
0·0001	,,	0·001	3·0
0·0001	,,	0·01	5·0
0·0001	,,	0·08	8·25
0·0003	Calcium nitrate	—	0·83
0·0003	,,	0·001	2·25
0·0003	,,	0·01	8·50
0·0003	,,	0·04	8·50
0·0003	,,	0·09	9·17
0·0003	Aluminium nitrate	—	0·83
0·0003	,,	0·0005	8·0
0·0003	,,	0·0025	12·5
0·0003	,,	0·01	?19·0

Thus the extent of the antagonistic action is dependent both on the concentration of the electrolyte and on the valency of the latter.
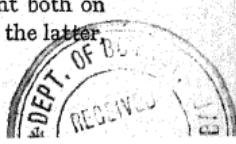
the higher the valency the greater the antagonistic effect, while
Szücs also found from experiments in which the concentration of
the electrolyte (calcium nitrate) was kept constant and the concen-
tration of dye varied, that the antagonistic effect also depended on
the concentration of the dye. The time required for the cells to acquire
a standard intensity of colour is not inversely proportional to the
concentration; it was found instead that the product of time and
concentration of dye in the external solution increased with increasing
concentration of dye. But no doubt the relation between time
required for the absorption of a definite quantity of dye and the
concentration of the dye in the solution, will depend on the quantity
of dye selected. The inadequacy of this method of investigation has
already been emphasized in an earlier chapter (Chapter x) and need
not be further stressed here.

That the retardation of absorption of dye brought about by the
presence of electrolyte is not due to an action between the dye and
electrolyte outside the cells, becomes evident from a number of
considerations. Practically the same concentrations of potassium,
calcium and aluminium nitrates are required to precipitate methyl
violet, so it would appear that the action of these three salts in
bringing about molecular association of the dye must be approxi-
mately the same in each case, and consequently the coefficient of
diffusion of the dye would be approximately the same in every case.
Again, it was shown that yeast cells become stained with a 0·005
per cent. solution of neutral red in less than 10 minutes, while in
the same solution containing aluminium chloride not until after the
lapse of 48 hours. If this were due to molecular association and
consequent reduction in the coefficient of diffusion, it would mean,
assuming Herzog's formula (see Chapter IV), that the molecular weight
of neutral red in presence of aluminium chloride is more than
2·3 × 10⁷, which is obviously an impossibly big magnitude.

A consideration of the results collected in Table XLVI makes it
clear that diluter solutions of electrolytes are relatively more active
in antagonising the entrance of the dye than more concentrated
solutions. According to Szücs his results with potassium nitrate agree
well, and those with calcium nitrate and aluminium nitrate approxi-
mately, with the equation

$$t = aC^{\frac{1}{n}}$$

where C is the concentration of salt and t the time taken for the dye
to enter. Because of the similarity of this expression to the adsorption

equation, Szücs concludes that the extent to which the intake of dye is prevented depends on the adsorption of electrolytes by the protoplasm.

Further investigations on this question have been made by Endler (1912 *a*) who used only truly soluble dyes, particularly methylene blue, but also neutral red. Among the species employed were *Ulva lactuca, Nitophyllum punctatum, Spirogyra* spp. and *Elodea densa*. Endler's results deviated somewhat from those of Szücs. With a large number of chlorides and sulphates added singly to a solution of dye, he found that the rate of intake of dye was increased with increase in the concentration of the electrolyte present, but with further increase in concentration of the electrolyte the intake of dye decreased. Thus, with *Elodea densa* in a solution of 0·05 per cent. neutral red it was found that the maximum intake of dye took place when the chloride or sulphate of a number of metals was present in a concentration between 0·01 M and 0·05 M. Similar results were obtained with methylene blue, with which the order of inhibiting action of different salts was as follows: salicylate (complete prevention of dye intake with 0·001 M), aluminate (complete prevention of dye intake with 0·01 M) [citrate, tartrate], [chloride, sulphate], nitrate (recognisable uptake of dye with 0·4 M). Little difference was observed between the different salts of the same anions, with the exception that aluminium salts hinder the intake of dye more significantly than other salts examined.

Where the results of Szücs and Endler diverge, the explanation may be found in the fact that Szücs observed the time taken for a certain quantity of dye to be absorbed, while Endler was concerned with the total quantity of dye absorbed at equilibrium. Also the materials, both dyes and plant species chiefly used, were different in the two sets of experiments.

The action of a number of substances in influencing the intake of sulphonic acid dyes by perianth leaves of *Hyacinthus* has been examined by Collander (1921). He found the intake of cyanol and orange *G* was strongly hindered by a 2 per cent. solution of ether, although a 1 per cent. solution had only a little influence, while solutions containing 3 per cent. ether or more killed the cells in a short time. Chloral hydrate in concentrations from 0·5 to 1 per cent. also inhibited the intake of the dye, while 2 per cent. killed the cells in a short time.

Collander, using dyes in presence of phosphate mixtures of definite hydrogen-ion concentration, concluded that the hydrogen-

ion concentration greatly influences the intake of cyanol and orange G by perianth leaves of *Hyacinthus*, the intake being furthered by acidity and reduced by alkalinity. A 0·25 to 0·2 per cent. solution of ammonium carbonate completely prevented the intake of cyanol and orange G. These results fall into line with those of Harvey (1911) and Endler (1912 *b*) who found the reverse influence of acidity and alkalinity on the penetration of basic dyes. It is not clear how far these results are connected with the general phenomena of antagonism.

Szücs (1910) found that the rate of intake of a basic dye, methyl violet, or neutral red, is much reduced in presence of the acid dye congo red. This is attributed to the formation of a compound of the two dyes in the external solution: a compound which cannot enter the cell. The non-entrance of the dyes in such cases is obviously not comparable with the other cases already discussed.

Methods of making quantitative determinations of antagonism by comparing amount of growth in plants in different solutions under otherwise the same conditions, have been formulated by Osterhout (1914 *b, c, d*). While such methods are undoubtedly of importance from the point of view of the study of growth, they do not provide a means of determining intake or permeability under different conditions of composition of the external solution. One point of importance emphasized by Osterhout (1914 *d*) to which reference may be made is that the relative concentration of two salts in which antagonism is greatest is not affected by dilution or concentration of the mixed solution. This is certainly an important fact, but to state that this law of direct proportionality "is in reality Weber's law" (Osterhout, 1916 *c*) appears to me an unwarranted extension of the scope of Weber's law[1].

Exact quantitative data relating to the influence of one substance on the intake of another are for the most part wanting. The best available appear to be those of Szücs on the intake of methyl violet in presence of various inorganic salts; these experiments have already been described. It will be necessary to obtain a vast quantity of further data before Szücs' view that antagonism in this case results from adsorption of the inorganic salts by the protoplasm can be accepted, especially as Endler's experiments on the intake of methy-

[1] "Weber's law states that 'the just noticeable increase of a stimulus bears a constant ratio to the original stimulus,' or 'two stimuli, in order to be discriminated, must be in a constant ratio, the latter being independent of the absolute magnitudes of the stimuli.'" (Flack and Hill, 1919.)

lene blue and neutral red suggest that the phenomenon cannot be so simply explained.

Osterhout (1916 *b, d*, 1917 *c*) has formulated what he calls a "dynamical theory of antagonism" based ultimately on the assumption that "an accurate measure of antagonism is furnished by the electrical resistance of living tissues." It is found that substances are of two kinds, those like sodium chloride, which bring about a fall in the electrical resistance of the thallus of *Laminaria Agardhii* and other plants, and those which, like calcium chloride, bring about an increase in electrical resistance followed very shortly (often within 15 minutes) by a decrease in resistance, which proceeds, as in the case of the first group of substances, to a maximum when the tissue is dead.

Osterhout supposes that two processes are involved, one producing a fall in resistance, the other a rise. He assumes that these two processes can be represented by the simple scheme

$$A \to M \to B,$$
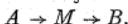
where the substance A breaks down to form an intermediate substance M which itself breaks down to form B. It is further assumed that the resistance of the protoplasm is due to the intermediate substance M ("a substance at the surface of the cell which offers resistance to the passage of ions" (Osterhout, 1917 *c*)), and that the resistance of the tissue is proportional to the quantity of M + a constant equal to the resistance of the tissue when dead.

It is assumed that in *Laminaria* under normal conditions in sea water, the quantity of each of these substances remains constant. On transferring the mixture to a solution of sodium chloride or calcium chloride, or a mixture of the two, it is further assumed that the velocity constants of the two reactions $A \to M$ and $M \to B$ are altered. Then, if the reaction $A \to M$ is more rapid than the reaction $M \to B$, M will accumulate and the resistance will be raised until the supply of A becomes exhausted when M will form more and more slowly, so that ultimately it will decompose faster than it is formed, when the resistance will fall.

Values can be selected for the velocity constants of the two reactions so that on the assumptions already made curves between time and quantity of the substance M present can be constructed which agree with the curves of resistance of *Laminaria* in sodium chloride, calcium chloride or any mixture of the two salts. It is then found that as the quantity of calcium chloride in the solutions increases, the velocity constants of both the actions

$A \to M$ and $M \to B$ first rise and then fall, the minimum value
naturally occurring in the mixture of salts in which the fall of
resistance is slowest.

To account for these changes in the values of the velocity con-
stants, Osterhout puts forward two alternative suggestions. According
to the simpler one, the rate of the first reaction is increased by calcium
chloride, which has a minimum influence when the molecular pro-
portion of this salt to sodium chloride is 4·76 : 95·24, that is, when the
proportions are such that the resistance falls most slowly, while the
rate of the second reaction is increased by sodium chloride, which
also has a minimum effect when the ratio of calcium chloride mole-
cules to sodium chloride molecules is 4·76 : 95·24. "It makes little
difference whether the value of the constant is increased by the salt,
the effect passing through a minimum, or diminished by the salt, the
effect passing through a maximum."

The alternative explanation supposes that the first reaction,
$A \to M$, that is, the formation of the substance to which the tissue
owes its resistance, is catalysed by calcium chloride, while both the
first and second actions are retarded by the presence of a substance
such as $Na_{20}XCaCl_{22}$ formed between sodium chloride, calcium
chloride and a constituent X of the cell, and the action is supposed
to be reversible. The quantity of this substance present is then
governed by the law of mass action and can be calculated from the
equation

$$\frac{C_{Na_{20}XCaCl_{22}}}{(C_{NaCl})^{20} \, C_X C_{CaCl_2}} = K,$$

where the symbols C_{NaCl}, C_{CaCl_2}, etc., represent the concentrations of
the various substances concerned, and K is a constant.

When the maximum quantity of this negative catalyst is pro-
duced there will be maximum antagonism. Now, according to
Osterhout, the position of maximum antagonism is independent of
the absolute concentrations of the antagonistic substances, while
the relative proportions of the salts giving a maximum quantity
of the compound $Na_{20}XCaCl_{22}$ will depend on the concentration. To
overcome this difficulty, Osterhout assumes that the negative catalyst
is formed at the surface, the sodium chloride being supposed to migrate
into the surface until the surface layer is saturated, so that with
increased concentration of the salts either inside or outside the cell,
the concentration in the surface layer remains constant.

It must be admitted that this is a rather tremendous super-
structure of theory built on the foundation of the changes in electrical

resistance of *Laminaria* disks in solutions of sodium and calcium chlorides. While the explanations may be credible they are, for the most part, speculative, and on this account they do not call for consideration here. It may, however, be said that to regard changes in resistance as dependent on, and proportional to, the change in quantity of one substance in the cell, appears to the writer, for reasons cited in the previous chapter, to neglect the complexity of the system involved.

(To be continued)

IMPERIAL BOTANICAL CONFERENCE, 1924

AT a representative meeting of botanists held recently at the Linnean Society's rooms it was decided to hold a Conference of British and Overseas botanists early next summer on the occasion of the British Empire Exhibition. An Executive Committee was appointed with Sir David Prain as Chairman, Dr A. B. Rendle as Treasurer, and Mr F. T. Brooks as Hon. Secretary. An invitation to attend the Conference has been sent to all Overseas botanists. It is expected that the Conference will include discussions upon modern aspects of Systematic Botany, Ecology, Morphology, Plant Physiology, Genetics, Plant Pathology, and upon the best means of effecting interchanges of students and staff between different parts of the Empire. Two meetings of the Executive Committee have now been held and the date of the opening of the Conference has been fixed for Monday, July 7, 1924. The meetings will all be held within the week, and excursions will be arranged for the weekend and the beginning of the following week.   F. T. B.

THE BRACTLESS INFLORESCENCE OF THE CRUCIFERÆ

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(With Plate III and 9 figures in the text)

I N a recent communication[1] dealing with the significance of certain superficial features exhibited in the stems of a large number of the higher plants and occasionally also in the hypocotyl, it was shown that in anatomical evidence of the kind indicated we have clear proof that the entire surface of the shoot axis is composed of the extensions of the leaf areas below the point of exsertion—to use the very appropriate term recently employed by other writers. For in some species these downward extensions, which are fused internally with the axis tissue proper and laterally with each other, have their *potential* edges demarcated by visible anatomical indicators such as hair, ridge and colour lines. At the same time it was realised that certain well known exceptional constructions might appear not to be covered by the above generalisation, and to need further investigation. One such case would seem to be furnished by the so-called bractless raceme of the Cruciferæ, of which we have a typical example in the Stock (*Matthiola incana*). But though the (apparently) bractless condition is a very general feature in Cruciferous inflorescences, it is to be noted that this apparent total suppression of all bracts is by no means universal in this family. Many cases are enumerated by Godron[2] where larger or smaller bract structures have been observed, and according to this author a bract, generally rudimentary but sometimes more or less well developed, is quite frequently present. He further notes the not infrequent occurrence in the inflorescence of decurrent ridge lines from the bract margins and midribs similar to those starting from the leaf insertions, and comments upon the fact that these features are sometimes observable *even when the bract is undeveloped*[3]. (Viewed from the standpoint of the "Leaf-skin" theory, these contour lines no longer present a difficulty, see foot-

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[1] The Leaf-skin Theory of the Stem, *Annals of Botany*, 36, p. 135, 1922.
[2] *Ann. Sci. Nat.* 2, p. 281, 1864.
[3] See also Norman, *Ann. Sci. Nat.* 9, p. 124, 1858.

note 1, p. 150.) Wretschko[1], Eichler[2], Baillon[3], Masters[4] and others also state that bract rudiments occur exceptionally in various Cruci- feræ and sometimes attain full development. There is thus a good deal of evidence to show that in a number of Cruciferous species, at least in some circumstances, a region of the inflorescence axis may show a normal construction quite in accord with the conception of the leaf-skin referred to above. It therefore becomes necessary to examine more closely into the actual conformation where the bract is seemingly absent. For this purpose the Garden Stock offers, as we shall see, particularly suitable material. Specific mention of this plant in this connection is made only by Eichler[5], who cites *Matthiola annua* as a case where the bracts are wanting, and Masters[6] who, on the other hand, includes *Matthiola incana* among the species listed as occasionally having bract structures present. He, however, gives no further information.

The normal sequence of shoot development in the Stock is as follows. The main axis, after giving rise to a close rosette of numerous spirally arranged leaves, produces well-developed internodes so that the leaves formed later stand far apart, and finally terminates in an apparently bractless raceme. The axillary bud of the lower leaves on the main axis remains undeveloped. In the succeeding region the buds give rise to a leafy branch which in turn terminates in a raceme. The first two leaves on these lateral stems are usually placed close together and opposite to one another at the extreme base, their position in relation to the subtending leaf on the main axis corre- sponding with that of a pair of bracteoles to the main bract. In the lower branches these two leaves are succeeded by several others placed singly at considerable intervals. As we ascend the plant, the number of leaves so borne diminishes, until perhaps the basal two alone are formed: or, even these two may be wanting, the lateral axis developing flowers straightway. If secondary lateral axes are produced, similar relations hold good. Although in the majority of individuals, as already stated, the pedicels appear not to be sub- tended by a foliar structure, one can often find individuals here and there in a large culture in which a normal leaf of larger or smaller size occurs immediately beneath and subtends the lowest or next flower in the raceme (Fig. 5), though the succeeding flowers are not thus accompanied. In others again a small subulate process may be

[1] *Sitzungsberichte d. k. Akad.* Wien, Bd. 58, 1, p. 211, 1868.
[2] *Flora*, p. 535, 1865.   [3] *Hist. Plant.* 3, p. 181, 1872.
[4] *J. Linn. Soc.* 14, p. 391, 1875.
[5] *Loc. cit.*   [6] *Loc. cit.* p. 394.

formed beneath some of the lower flowers at varying levels from the base of the pedicel upwards (Figs. 3–4), a faint decurrent outline indicating the delimitation of the downward extension below the exsertion point (Fig. 4). The indeterminate position is due to a prolonged fusion of the bract process with the pedicel which it subtends, and the consequent carrying up of the exsertion point. [It may be noted in passing that some amount of fusion of the lowermost pedicels with the main axis is also of common occurrence, and was evidently present in the specimen of the Stock used for the illustration in Bessler's *Hortus Eystettensis*[1].] In the hoary type these processes are easily overlooked owing to their being partly imbedded in the felt-work of hairs (Figs. 10–11), but in glabrous individuals they stand out conspicuously (Figs. 12–13). In a glabrous plant which has the constitution CRH the otherwise hairless process may bear at its apex one characteristically branched hair like the ordinary foliage leaves in this strain[2] (Fig. 13). The process is clearly the very much reduced free region of a subtending leaf (bract). It contains no vascular tissue and but little chlorophyll, and constitutes merely a very simple hydathode, for two or three water pores are generally present. Except for this latter feature, it is remarkably like the rudimentary leaf structures in *Psilotum triquetrum*. Immediately below the exsertion level the undisturbed vascular ring of the stem lies slightly further from the surface than at other points of the circumference, owing to the slight increase in bulk of cortical tissue forming the downward continuation of the process. Such bract-processes are not restricted

[1] Classis æstivalis, fol. 33, 2, 1640.
[2] See *J. of Genetics*, 10, p. 159, text-figure 1, also Pl. VIII, Figs. 7 and 8. C and R denote the two factors required to produce coloured sap. H = one of the factors for hoariness.

DESCRIPTION OF TEXT-FIGURES ON p. 152.

Figs. 1–2, *Cheiranthus Cheiri*. Fig. 1, a flowering shoot with a bract-process accompanying the lowest flower; the downward extension of the process can be traced to the mid-axil of the fifth leaf below as usual in a ⅖ arrangement. [The similar demarcation lines from the foliage leaf insertions are not shown in the drawing.] Fig. 2, portion of the same showing the bract-process more highly magnified. N.B. Figs. 3–9, *Matthiola incana*. Figs. 3–4 show a bract-process accompanying the flower and fused for some distance with the pedicel. Fig. 5, the lowest flower in the raceme subtended by a foliage leaf. Fig. 6, young inflorescence accompanied by a sickle-shaped leaf which is half foliage leaf, half sepal in form. Fig. 7, a sickle-shaped leaf more highly magnified showing the crinkled membranous border characteristic of a sepal along the concave edge. Fig. 8, a shoot bearing a flower accompanied by a "sepal-bract." Fig. 9, a "sepal-bract" overarching a young inflorescence and thus causing the axis to become curved.

to the bottom flower on the axis. They may be detected perhaps
up to the fifth or sixth, but I have never seen them attain develop-
ment the whole way up the inflorescence[1]. Similar non-vascular
bract structures are also frequently found in the Wallflower (Figs.
1–2). Again in some cases, in addition to a subtending leafy bract
an asymmetric sickle-shaped leaf structure may be present in the
position of one member of the basal leaf pair (Fig. 6). This peculiar
shape was at first attributed to injury, but on closer inspection it
was noticed that the concave margin of these structures always
differed from that of the convex side in having a transparent, and,
in coloured forms, often a tinted membranous border (Fig. 7). The
border and the upper surface of the concave half in hoary as well
as in glabrous strains was devoid of hairs. This combination of
characters showed at once that we are dealing here with a structure
which is half leaf and half sepal[2]. The sepaline sector being capable
of only a very limited longitudinal extension, the whole structure
becomes curved. Sometimes the sepaline border may be developed
for only quite a short distance and then cease; the sepal-bract then
presents an appearance as though a piece had been bitten out on
one side. In other cases the sepaline modification occurs along the
whole of *both* margins, and we then get a symmetrical boat-shaped
bract identical in form and appearance with the sepals proper
(Figs. 8–9). When the complete transformation occurs, the whole
young raceme may occasionally be found curved into a bow, for the
sepaline bract is unable to keep pace with the growth in length of
the axis, and the boat-shaped tip overarching one of the flower buds
acts as a grapnel and holds the whole short apex down. Where, as
is often the case with the upper branches there is but little elongation
of the lateral axis and the basal leaf pair is followed at once by the
flowers, both of these leaves may be thus modified. The normal
floral arrangement is not as a rule affected by such transformations,
the full complement of four sepals being present. If however the
calyx follows upon a sepal-like bract without any appreciable inter-
node, one of the true sepals may be lacking. This assumption of
sepaline characters by the bracts was observed by Engler[3] in a
monstrous form of *Barbarea vulgaris*, in which several flowers had

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[1] In *Iberis semperflorens*, on the other hand, bracts may attain develop-
ment in the upper part of the inflorescence and be undeveloped in the lower
(see Eichler, *loc. cit.*).
[2] The leaf in a hoary stock is hairy on both surfaces, the sepal is glabrous
on the upper (inner) face and hairy beneath.
[3] *Flora*, p. 450, 1872.
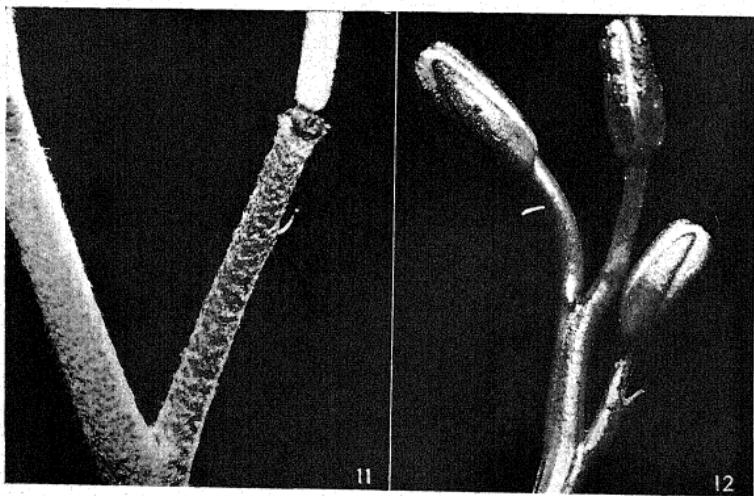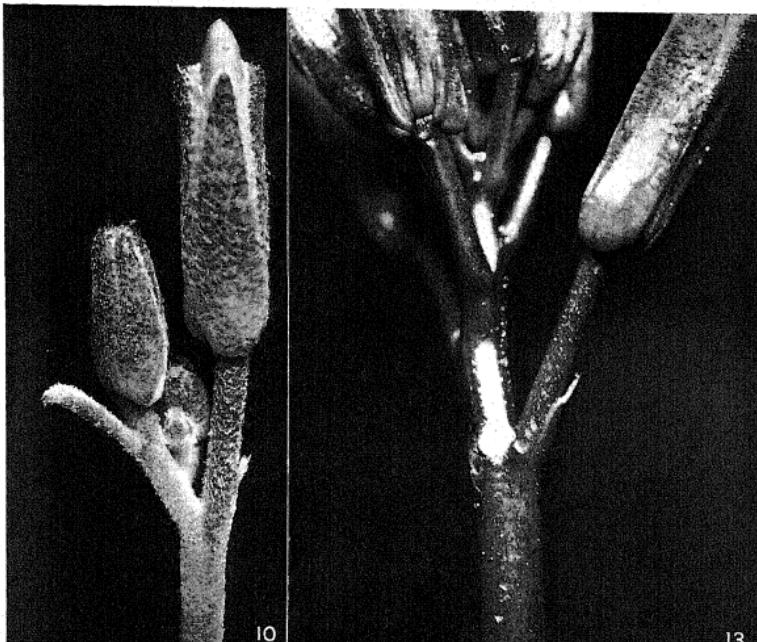
less than four sepals. But the normal structural balance in this case seems to have been altogether upset, the plants being abnormal throughout in other respects.

At present we can only speculate as to the immediate cause of this morphological instability, for the modifications described above occur apparently quite irregularly, it being impossible to predict in which individuals they will be found. But we can scarcely go wrong in supposing that the same process of reduction which has undoubtedly taken place in the gynœcium[1] and probably also in the andrœcium of Cruciferæ has likewise had its effect on other parts of the inflorescence, and that in the precursors of the existing representatives of this family each flower was furnished with a fully developed bract. As the process of reduction set in and progressed, we can conceive that the free lamina of the bract ceased to be developed, the basal extension alone being present and remaining unaltered, and thus the raceme came to assume its present bractless appearance. The great variability exhibited by the occasionally-formed bract lamina in the Stock and its irregular occurrence points rather to a *re*-appearance under favourable conditions of a lost structure than to a last flicker before final *dis*appearance of a member generally exhibiting the uniformity and constancy indicative of strict inheritance but little affected by varying conditions.

DESCRIPTION OF PLATE III

Figs. 10–13, *Matthiola incana*. Fig. 10, flowering shoot of the hoary type showing a small hairy bract-process accompanying the oldest flower and a small leaf subtending the next; both are exserted some distance up the pedicel. Fig. 11, another specimen showing the exceptional case of a bract-process in a fully hoary type glabrous except for the terminal hair; the absence of hairs elsewhere on the process may be due to the extreme tenuity of the structure. Fig. 12, flowering shoot of a glabrous plant with smooth bract-processes to the two lowest flowers. Fig. 13, another specimen; the bract-process bears a branched hair at its apex (see text).

CONCLUSIONS

1. The description of the Cruciferous inflorescence as "bractless" must be understood to mean that it is only the free exserted region of the bract which has undergone suppression, the basal extension being still formed and clothing the axis with a "leaf-skin" in the same manner as if the region above the exsertion level had attained full development.

[1] The evidence for reduction in the gynœcium forms the subject of a separate paper now in the press.

2. The fact that foliar organs functioning as bracts sometimes occur in species which typically are bractless in this sense, and that even in one and the same species they may assume very varied forms, suggests that in such cases the bracts were in the first instance reduced to their basal extensions.

3. But that after this stage in the phylogenetic history has been reached, a favourable conjunction of circumstances may still allow of the reappearance of the exserted portion, hereditary control being then inoperative or subordinated to the influence of varying conditions. Thus in *Matthiola incana* the occasionally-appearing free portion may take on the form of a foliage leaf, a sepal, a structure half leaf, half sepal, or a small non-vascular process constituting a simple hydathode.

4. This sequence of reduction followed by occasional reversion is parallelled in most striking fashion by the phylogenetic history of the Cruciferous gynœcium, which is dealt with elsewhere (see footnote 1, p. 155).

For the drawings reproduced on p. 152 I am indebted to Miss D. F. M. Pertz to whom I here tender my most grateful thanks.

The expenses incurred in connection with the work have been defrayed in part by a grant from the Royal Society.

AN APOCARPIC PLANT OF THE RED CAMPION (*LYCHNIS DIOICA* L.)

By R. SNOW

(With 4 figures in the text)

THE plant described in this note was found flowering in a hedge-bank near Exeter in September, 1922.

The petals were vestigial or lacking, and the plant being carpellary, only minute rudiments of stamens were present. The carpels, however, were, in most of the flowers, entirely free, and 5 to 8 or more in number. They bore freely exposed ovules on their adaxial surface and terminated in stigmas of normal appearance (fig. 1). In a few flowers, a central syncarpous ovary of 2 or 3 carpels was surrounded by a ring of free carpels. The calyx was normal.
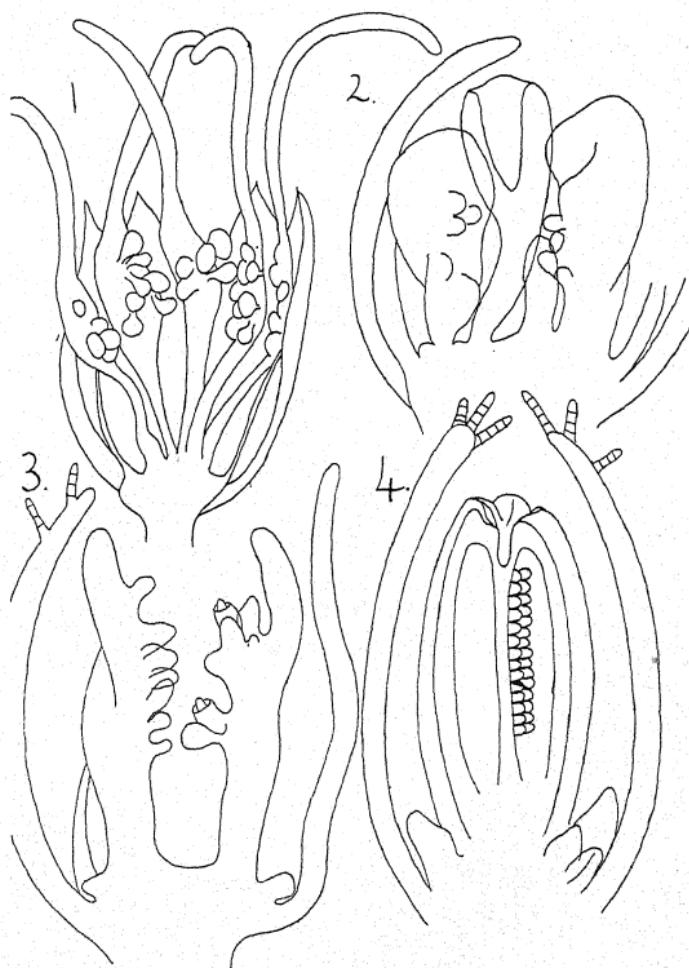
Fig. 1. Abnormal flower in elevation.

Fig. 2. Longitudinal section of very young bud of abnormal plant, showing
ovules just arising.

Fig. 3. A slightly older bud of the same.

Fig. 4. Bud from a normal carpellary plant, of about same age as (3), with
stigmas not yet formed.

The plant was brought into the garden, and in May of this year (1923) has flowered again with flowers of the same abnormal type. It has not been found to set any seed, but will be further tested.

In Penzig's *Pflanzenteratologie* (1921) no similar abnormality is described for *Lychnis dioica*, though reference is made to the occurrence of "several rings of carpels...in various *Sileneæ*" (Vol. 2, p. 138).

An interesting floral abnormality of a different kind is also described by Buchenau (*Ber. d. d. bot. Ges.* 21, p. 417 (1903)) for a plant of *Lychnis dioica*, under synonymy of *Melandrium rubrum*.

THE PROGRAMME OF SECTION K AT THE FORTHCOMING MEETING OF THE BRITISH ASSOCIATION, LIVERPOOL, SEPTEMBER 12—19, 1923

A VERY large number of titles and abstracts of papers intended for communication at this meeting have been received by the organising committee, and it has been no light task to fit as many of them as possible into the available time. The work to be communicated ranges over a large part of the vast field of modern botany, and, as usual, an effort has been made to keep the papers dealing with related topics as far as possible together, so as to avoid the constant distraction of the interest of the audience from one subject to another, though it has not proved practicable to group all papers in this way.

The President earnestly hopes that all readers of papers will remember that they are not addressing an audience exclusively composed of specialists fully instructed in the technicalities of their special branches of research, and that each author will take pains to be thoroughly intelligible to all botanists. It is legitimate for an author to assume that his hearers possess a good botanical training and a keen interest in the subject as a whole, but it is most undesirable that the Sectional meetings of the British Association should cease to be

intelligible except to different groups of people engaged in highly specialised departments of research. The occasion of the annual meetings of Section K forms an opportunity for every botanist to learn what is being done in branches of the subject other than his own, but he cannot do this with any completeness unless the author will put himself in the place of his audience. This result can be achieved in the great majority of cases without any sacrifice of substance.

The plan followed in arranging the papers has been to allot half-an-hour (in a few cases somewhat longer) to each author, including time for discussion. The author can decide for himself how much of his time he will devote to expounding his results and how much he will leave over for comment or criticism by others. But at the end of the allotted time it will be necessary to call on the author next on the programme. The necessity of interrupting what may be a profitable discussion is regrettable, but with a very full programme it is impossible to allow any considerable latitude without unfairness.

The President's address, which will be delivered at the outset of the sectional meetings, at 10 o'clock on Thursday morning, will be devoted to a consideration of the changes in our outlook on botany during the last few decades, the causes of these and their implications for the subject as a whole. The rest of the morning will be occupied by miscellaneous papers by Miss E. R. Saunders, Prof. H. H. Dixon and Mr N. G. Ball, Prof. Neilson Jones, and Dr M. C. Rayner. The afternoon will be devoted to three papers by Dr F. G. Gregory, Mr C. Hunter, and Mr F. G. Henderson, on the effects of light, temperature and CO_2 concentration on various functions of the plant. Mr S. G. Jones will speak on the life-history of *Rhytisma*.

Friday morning will be occupied by an address on Oxygen and Respiration by Dr F. F. Blackman. This will be followed by papers on the effect of electricity on plants, in which Prof. V. H. Blackman and members of his school will take the leading part, and by miscellaneous papers. The afternoon will be principally devoted to papers on recent work on chromosomes by Major C. C. Hurst, Miss Ferguson, Miss Campin and Miss Rees.

Saturday will be occupied by a field excursion, the place being not yet definitely settled.

Monday morning will be devoted to morphology. Dr D. H. Scott will open with an account of the Early History of the Stele, Prof. Lang will follow on the Organisation of the Plant in the Vascular Cryptogams, and Prof. McLean Thompson will deal with Developmental Morphology. Dr E. N. Thomas will describe observations on

the seedling anatomy of the Sapotaceae. In the afternoon Dr Gelston Atkins will deal with seasonal changes in sea water and their relation to plankton, Prof. Seward with the Cretaceous flora of Greenland, Prof. Thoday with *Passerina* and its geographical distribution. At 5 o'clock Dr W. L. Balls will give a popular lecture on Cotton.

Tuesday morning will be mainly devoted to a joint discussion with Section M (Agriculture) on Virus Diseases of Plants. Dr Paul Murphy will open, and will be followed by the distinguished Dutch authority Prof. H. M. Quanjer, and then by Dr W. B. Brierley, Mr T. Whitehead, Mr Holmes Smith, Mr Kenneth Smith, and others. In the afternoon there will be an excursion to the West Lancashire sand dunes.

Wednesday morning will be devoted to the Effect of Soil Sourness on Plants. The President will give a brief general account of the subject, designed for the orientation of members of the section to whom it is unfamiliar, and he will be followed by Dr E. J. Salisbury, Dr N. M. Comber, Dr W. H. Pearsall and Prof. J. H. Priestley. Different views of the essential factors at work will be put forward, and it is believed that an interesting and lively discussion will be secured.

THE

NEW PHYTOLOGIST

VARIATION AS AN ORGANIC FUNCTION

By C. W. SOAL, B.A.

I. Many controversies have centred round the relative importance
of the various "organismal" and "environmental" factors in evolu-
tion, the Lamarckian and mutationist theories differing markedly in
this respect. It is suggested in this paper that these differences are
due in part to certain limitations in the more fundamental concepts
of current biology, and that an alternative and perhaps more empirical
method of approaching the subject may tend to eliminate them.

The biologist customarily regards the organism as a material
structure, comprising an aggregate of characters, which is more or
less sharply defined spatially from the rest of the material universe
or environment by virtue of its peculiar properties. It is usual more-
over, for physiological reasons, to associate these properties more
closely with a definite portion of its structure—the protoplasts of
the cells. The organism thus becomes, as it were, a skeleton of "living"
matter surrounded by an unspecialised "primary" environment and
interpenetrated by a specialised "secondary" environment—the non-
living cell-sap, lymph, cell-walls, etc.—a conception which, while
pushing back further the dualism between organism and environ-
ment, does not resolve it. Both these assumptions are however
purely arbitrary conveniences. During the organism's life there is a
constant flux of matter through various physiological channels. The
protoplasts are also themselves organised structures, being differ-
entiated into cytoplasm, chromosomes, etc., and these in turn into
a variety of chemical substances variously distributed, some of which
at least are common to other parts of the organism, or even to the
primary environment. There is no logical reason, therefore, why the
process of localisation should not be carried further by the postula-
tion of subordinate centres of activity within the cell interpenetrated
by a "tertiary" environment, and so on indefinitely.

The physiological theory that the specific properties of the organism are a chemical function of its germ-plasm is, moreover, not really an induction from biological facts. What we actually observe in development, heredity, and variation is that certain material complexes—organs, tissues, cells, chromosomes, etc.—*change* in different ways, often periodic and variously correlated in constant fashion. Empirical biology is the study of these changes. The actual matter of the germ-plasm obviously does not persist during somatic life, since all, or practically all, the somatic protoplasm is derived ultimately from the primary environment in the course of development. The only constant factors are the co-ordinated groups of changes that we term cell-division, and cell-development, which give rise to definite recognisable types of cell structure, whether in the form of chemical compounds or larger material aggregates. The germ-cell is simply an observed link in a particular periodic series of *biological events* in some way causally related, and we must ascribe its specific properties primarily to this fact.

It is contended here that the physico-chemical standpoint, whether ultimately justifiable or not, is at present of no value as a working theory, since the phenomena with which empirical biology deals are not obviously physico-chemical in character. It is a facile generalisation that may indeed often act as an incubus rather than an asset in biological investigations, as will be subsequently shown, by leading to an uncritical confusion of different classes of phenomena that are qualitatively distinct. It is suggested therefore, that it is desirable in the present state of knowledge to devise a working theory of development, heredity, and variation framed in other than physico-chemical terms.

From this point of view we shall find it more consistent to discard altogether the dualistic conception of organism and environment, at least in the sense of implying spatial definition. Biological phenomena are regarded simply as changes in the material environment of a particular quality, that are variously correlated in groups and sequences. We must at present simply accept these correlations as observed facts; they form the inductive basis of our science. Any sequence of environmental changes that is sufficiently constant and distinct may be referred to an *organic activity*—without prejudice as to its ultimate nature. It is simply some particular constant aspect of the organism's behaviour in the course of development, the organism itself thus becoming a co-ordinated system of activities. An activity is always associated with a series of *energy-reactions*, but

it may or may not be more than the mere summation of such a series. It is a *unity*, co-ordinated in time and space, that has definite form and direction. Thus in every organism cell-division constitutes a primary activity. It is a type of organismal behaviour that we can recognise as being at once generally constant and qualitatively distinct, although we cannot at present give it any interpretation in physiological terms.

Cell-division is, however, by no means an isolated or sporadic phenomenon. In the process of ontogeny beginning with the first segmentation of the zygote, the successions of cell-divisions are co-ordinated in a number of parallel but interdependent sequences which give rise to the somatic morphological characters at every stage of development. The character of these sequences, and the way in which they are mutually related are also fundamental aspects of the organic activity-system.

As a somewhat more complex example, functional adaptation is a distinct type of activity. When for instance we observe that a plant responds to an increase in the intensity of sunlight by a variety of distinct and apparently co-ordinated bodily movements having the effect of reducing transpiration, we simply recognise certain changes in the environment-state of a particular familiar quality.

In the same way, in the higher organisms, instinctive and intelligent behaviour are distinct types of activity. Their psychological aspect, as inferred by analogy with our own subjective experience, does not primarily concern the biologist *qua* biologist. From the biological standpoint instinctive and intelligent acts are essentially a part of the process of somatic development, differing only qualitatively from cell-division, tissue-differentiation, and internal bodily readjustments that are the normal accompaniment of every individual life. There is however the important difference that the environmental changes by which these higher activities are recognised and defined are for the most part *external* to the body of the organism in the ordinary sense. In this category we should place not only co-ordinated bodily movements, but also the various external structures which result from the instinctive and intelligent behaviour of the higher animals, such as the web of a spider, the mud cells of the mason bee, the nest of a bird, the lodge of a beaver, or even a machine constructed by man. These "external characters" are essentially a part of the organism's morphological structure, and the energy-reactions with which they are associated are an integral part of its physiological economy. They are definitely specific, and may, or may not, show a

greater range of individual fluctuation than the ordinary bodily characters to which the biologist customarily confines himself.

This difference between internal and external characters necessitates, however, that we should define the sense in which we use the term "environment" a little more precisely. It is proposed to retain the distinction between the unspecialised *primary* environment outside the organism, and the specialised *secondary* environment which comprises *all the matter within the sphere of its activities.* In plants the secondary environment will be practically all included within the morphological body. In the much more complex case of an insect colony however, it will also include all material structures associated with the life of the colony that have resulted from environmental changes brought about by the activities of the individual insects, such as the nest, cocoon-cases, and external food supplies.

Activities are thus seen to be definite aspects of the life of the organism of which we have an empirical knowledge. They are *events* in the phenomenal world having properties of their own, that place them in a unique category. Even if it be ultimately true that an activity is only a sequence of energy-reactions physically determined, the more empirical method of investigating biological problems advocated here has at least the advantage that it enables us to deal more readily with very complex phenomena by utilising the fact that we find certain natural groupings ready to hand. It may be, of course, that this method will afford only approximations to the truth, whereas a fuller and more accurate interpretation of life processes could be given by a complete physiological knowledge. But as there is at present no possibility of such a complete physiological knowledge, it may be legitimate and useful to employ more empirical data as the basis of a provisional hypothesis.

The relation of this view of biological phenomena to the ordinary conception of structure and function is a simple one. At any moment in the organism's life there is present a certain definite environmental configuration. This consists of a number of elements, some of which are primary (air, light, water and other organisms), and some secondary, *i.e.* having special qualities or dispositions resulting from the past activities of the organism in question. A structural character, such as a tracheid of a plant, is a group of secondary environmental factors that remain relatively constant during an appreciable period. The function of such a structure (in this case water-conduction) is a persistent type of energy-reaction in which those particular environmental factors are involved. As a somewhat different illustration,

in the case of a colony of ants, the various galleries, chambers, food stores, etc., in the nest, and also the cultures of aphides, fungi, etc. which the ants control are all persistent secondary environmental factors, and therefore *structural characters* of the colony, regarded as a biological individual. It is possible, of course, to imagine a type of fluid organism exhibiting a very complex behaviour in relation to the outside world, but in which no obvious persistent environmental factors were recognisable. Fortunately for biological science, this is not so in most cases.

One of the principal distinguishing characteristics of the organism regarded from any point of view is its *individuality*. From our standpoint, this is recognised by the fact that its activities are functionally integrated. By integration is not implied any physiological mechanism, but simply the observed fact that the organism behaves as a unity, as revealed by the existence of definite and constant types of correlation in various environmental changes. When for instance a plant is given a restricted water supply, it may check vegetative growth and hasten the process of flower and seed formation. This is a very complex phenomenon, involving the interaction of several distinct trends of behaviour that permeate the whole organism. It can be explained most simply on the assumption that the various activities (*i.e.* the sequences of events which characterise the processes of growth and reproduction) are functionally integrated, and that the whole complex has the capacity of regulating the expenditure of the accumulated energy of the organism through various channels in different circumstances.

Functional integration is moreover not confined to single organisms, but may also be an observed characteristic of the relation between different individuals. This is probably true, for instance, in certain cases of symbiosis, such as that between algae and fungi in lichens. Here also there is an observed co-ordinated behaviour in metabolism, growth, and asexual reproduction, in relation to a variety of external conditions.

The integration of activity-systems, whether in the same or different individuals, is always associated with some kind of environmental-reaction[1], but it is important to observe that the two phenomena are quite distinct. Two or more different organisms may interact physiologically, and even interpenetrate each other spatially,

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[1] A generic term for any kind of energy-reaction associated with the life of the organism, including those which are *internal* or *physiological*, and those which are *external* or *economic*.

without exhibiting any co-ordinated behaviour *in relation to a variety of different circumstances.* Parasitism affords one of the best illustrations. The parasite and the host simply modify each other's environment, just as two plants growing side by side may deprive each other of light or moisture. There are, however, many cases in which such accidental or non-co-ordinated reaction may be mutually beneficial, as for instance in some types of symbiosis. In the relations between various orchids and the fungus *Rhizoctonia,* there has been found to be a continuous gradation from parasitism (harmful association) to symbiosis (beneficial, and in some cases necessary association) according to virulence or biological type of *Rhizoctonia* with which the host has been infected. This kind of symbiosis does not imply functional integration. The same is true of the association of nitrifying bacteria with the roots of leguminous plants. These phenomena simply mean that in the course of evolution the host has developed a system of physiological economy, in relation to a particular type of primary environment in which a certain kind of fungus is a necessary factor. They are examples of mutual adaptation, not essentially different from that between various species in an ecological group[1].

There are, however, other complex and indubitable examples of functional integration in which the activities of different individuals are involved, as for instance in colonies of social insects. In this case also, integration of activities is accompanied by physiological reactions, some of which are observed and others only presumptive (*e.g.* the existence of complex stimuli and inherited reflex nervous mechanisms), but it is not from such reactions that the unity of the colony is inferred. We recognise this unity by the way in which the instinctive acts of the different individual insects are co-ordinated in the many diverse circumstances that arise in the course of the colony's life. In the development of human society there is a still more complex example, where the activities functionally integrated include both instinctive and intelligent behaviour.

We have endeavoured to establish in the preceding discussion that functional integration is a unique biological phenomenon. In the individual organism the activities are always integrated; but in the relations between different individuals there may be environ-

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[1] In some cases however one partner may "control" the other, as in certain *insect-bacteria* associations where the female has special organs for infecting the ova. The fungus has in this case become incorporated in the *secondary* environment of the insect, and the reactions between them are only accidental so far as the fungus is concerned.

mental reactions with or without functional integration. It is possible of course to *assume* an ultimate physiological interpretation of even the most complex examples (such as insect colonies and human society). But such an assumption does not help us to understand the proximate significance of these various phenomena in development and evolution. On the other hand, to describe the latter in psychological terms fails to correlate them with the inferior activities of the organism with which they are intimately associated. From our standpoint, all cases of functional integration are qualitatively similar and must be regarded as biological phenomena of one type, which may be defined in terms of the co-ordination of activity-systems here adopted.

In the relations between different organisms there may, however, be a continuous gradation from the extreme cases of physiological reaction with no integration (parasitism or mutual adaptation), to the most highly integrated social groups. The various examples of colonial algae, polyps, etc., probably occupy an intermediate position. A simile will perhaps serve to make this important distinction clearer. The life of an organism, or of several associated organisms, may be compared with a skein of variously-coloured threads. Each thread represents an activity (*i.e.* some particular sequence of biological events), while the cross-section of the whole skein represents the environmental reaction-complex at any moment. The degree of functional integration then becomes represented by the constancy in the relative position of the different threads towards each other at different positions in the skein. If it is found to consist of a single twisted bundle of threads, it represents a biological individual; if a number of separate interlacing bundles which nowhere run parallel for any appreciable distance, it represents different reacting individuals the activities of which are not functionally integrated. There may of course be an indefinite number of intermediate cases.

Conversely, there are markedly abnormal types of behaviour on the part of biological individuals that must be ascribed to the functional dissociation of stable activity-systems which have been built up in the course of evolution. As examples may be mentioned: cytological aberrations in particular organic tissues; fœtal monstrosities; and the more complex cases of functional mental diseases in the higher animals and man. In some instances it may be possible to point out particular environmental factors as the proximate cause of the abnormality, but not in others. The interpretation in terms of functional dissociation is, however, applicable to all cases.

II. It is necessary now to investigate further the relation between the sequences of biological events which we have termed organic activities, and the energy-reactions with which they are invariably associated. Now whether the former are, or are not, capable of an ultimate interpretation in physiological terms, there is abundant evidence that they are always *limited* by the intrinsic physical properties of the primary and secondary environment. In general terms it may be said that throughout development the organism is always conditioned by the persistence of certain physical equilibria between its various reactions. We shall deal first with the operation of limiting factors in the secondary environment.

It is possible to ascertain some of the proximate secondary factors in somatic equilibrium owing to the persistence of certain types of reaction as "structure and function" through various periods of development, our present knowledge being most extensive in the case of adult organisms. The internal or physiological reactions always include some of the most important factors, which are also usually more fundamental from the phylogenetic point of view. The life of an adult mammal, for instance, is conditioned by the fact that it has acquired *somehow*, in the course of individual and racial development, a type of internal economy involving the interaction of various structural characters such as the nervous, vascular and glandular systems that remain relatively constant during an appreciable period. If any of these characters were changed, in some cases even in certain minute details (*e.g.* the chemical function of the erythrocytes), the animal would die unless there were at the same time some compensative readjustment in its physiological economy. The continued stability of the organism throughout ontogeny is dependent upon the fact that the changes which do take place in these physiologically important characters are mutually compensative in this respect. It must be carefully noted however, that these physical equilibria conditions do not actually determine the sequence of biological events, which must still be ascribed to a particular quality of the activity-system; they merely impose certain limitations within which possible sequences may occur[1].

In the higher types somatic equilibrium may be also largely conditioned by environmental reactions external to the body of the organism, as for instance by the efficiency of the various mechanical

[1] In the case of plants the proximate secondary environmental limiting factors are not so obvious, but they are present none the less; the function of the chloroplasts and the structure of the vascular system may be mentioned.

contrivances that insects construct for capturing and preserving food. The most complex example of all—human society—is characterised by the fact that this external economic machinery is of paramount importance, and upon its stability and efficiency the life of the majority of the members of a highly developed community is ultimately dependent. In all these cases, the persistence of the important structures and functions is due mainly to a particular series of instinctive and intelligent acts having followed each other in a definite order. There may indeed be a very large number of different *types* of physiological economy corresponding to different types of activity-systems, but in each case it is generally possible to discover certain stable reactions that are limiting factors in somatic equilibrium, and that can only vary within certain restricted limits without throwing the whole machinery of somatic life completely out of gear, unless there are compensative readjustments in other parts of the organism.

The character of the environmental reaction-complex is, however, also partly determined by the uncontrolled primary environment. And as this is a variable factor the maintenance of a stable and efficient organism necessitates a certain *elasticity* in the inherited sequence of events. This capacity for somatic readjustment with respect to variations in the primary environment is generally referred to as "functional adaptation," although the term "somatic equilibration" is perhaps a less contentious one. In the simplest cases, such as the compensative readjustments between root-absorption and transpiration in plants, or between the amount of assimilatory tissue in the leaves and the intensity of sunlight, the reactions involved are internal and physiological. The same is true of the slightly more complex example of the compensative hypertrophy of various glands in the bodies of animals when others known or presumed to have a similar function have undergone excision or atrophy. There are, however, in the higher types examples of ontogenetic readjustments, the mechanism of which is not so obviously physiological, as in the slight variations in the instinctive behaviour of insects conditioned by inconstant external factors, or in the most complex example of all, the intelligent acts of the higher animals and man. Another very specialised type of functional adaptation that cannot be discussed here is the non-rational changes in social conduct that are characteristic of the progressive development of human society, and which affect so profoundly the economic structure of the group.

Here again we are dealing with biological phenomena of one type. It serves no useful purpose to attribute the simpler cases of functional adaptation to the direct physiological effect of physical conditions upon the organism, as we cannot at present interpret the *normal* process of development in physiological terms. All that we are entitled to say on empirical biological grounds is that the limiting environmental factors having changed, the type of reaction-complex conforming to somatic equilibrium has also changed, but the actual observed sequence of events still remains an intrinsic property of the organism. In the most complex examples, there is, moreover, some evidence that somatic readjustments may take place independently of changes in the primary environment.

III. Let us now consider the bearing of the foregoing discussions on the problem of racial development. All evolutionary theories recognise that variations are transmitted through the medium of the germinal cycle in normal inheritance, but they differ in their explanation of how germinal variations originate. There are two main divergent points of view on this subject: (1) the Lamarckian theory of functional inheritance; (2) theories which rest upon the assumption that germinal variations are *spontaneous, i.e.* not in any way conditioned by the course of events in the rest of the organism. We shall consider first certain aspects of the second hypothesis.

Spontaneous variations are frequently classed as continuous and discontinuous, but a much more important difference is between those which are *fortuitous*, or individual in character, and *determinate* variations which are generally attributed to a specific property of the germ-plasm. The Darwinian theory, which ascribes specific divergence to a differential extinction of individuals in the struggle for existence, is equally applicable to minute continuous variations and to sporadic individual mutations of larger extent if they are hereditarily transmissible. It has been shown, however, by genetic experiments that the observed individual fluctuations which formed the original inductive basis of this theory are incapable of hereditary transmission, and therefore of having any cumulative effect upon specific type. The normal phenomena of heredity seem, moreover, to imply the existence of some stabilising factor in the germinal cycle which does not generally permit any serious modification in the properties of the cell. Whether this factor is regarded as an intrinsic "chemical stability" on the part of the germinal complex, or a particular "quality" of the activity of cell-division, it does not seem very consistent with the view that any and every property of the

cell is indefinitely variable. There is evidently a certain elasticity in the hereditary type which permits fluctuations within certain limits in successive generations without impairing the organism's capacity for reverting to a normal equilibrium position. These fluctuations are generally environmentally conditioned, but there is no necessity for assuming that this is always the case.

In the case of *determinate* variations however, since they are specific and not individual phenomena, the extinction of individuals will have no evolutionary importance, and natural selection will act only as an eliminatory factor in the reactions between different species. This will be equally true whether the variations in question are relatively large mutations which affect several characters simultaneously, the smaller "parvigrade" mutations confined to a single character, or the minute cumulative changes known as orthogenesis. The effect of hybridisation as an evolutionary factor (by effecting various combinations of different characters in bisexual inheritance) will also be obviously unimportant in the case of variations of this class.

We shall proceed to a criticism of the theory of determinate variation on more general grounds. Now so long as we regard the organism as a kind of static aggregate of characters corresponding to a geometrical pattern of factors in the germ-cell, it is of course possible to postulate that for every change in the germinal complex there will be a corresponding change in the somatic complex. This view has been elaborated by means of a chemical analogy in the "nucleus and side-chain" theory of germinal structure, corresponding respectively to the more stable ordinal and generic characters in the organism, and the less stable specific and varietal ones. It is supported also by an appeal to alleged correlations in the morphological structure of the germ-cell and the soma as revealed by cytological and genetic analysis, *e.g.* sex-characters and sex-chromosomes. But as we have seen, morphological characters are only symbols of persistent reactions peculiar to a particular type of physiological economy. The germ-cell has the dual function of initiating two distinct series of events—the germinal and somatic cycles—and it is the relation between these two series, and not between the geometrical configuration of the germ-cell and the soma at particular stages of development, that is of real importance.

We have also seen that somatic development is always *conditioned* by the persistence of certain physical equilibria throughout on togeny. The organism inherits *somehow* a particular type of physiological

economy, but whether it is workable or non-workable, efficient or inefficient, is mainly a physical property of the type itself. The same is true of the more restricted germinal cycle (in relation to its somatic environment). We know very little, it is true, about the proximate factors in germinal equilibrium which must be maintained in the succession of cell-divisions[1] along the germ-track, if a new germ-cell is to be produced. But we may presume that it implies a certain co-ordination of physiological reactions between the various complex proteins, lipoids, nucleic acids, etc., of the chromosomes and the cytoplasm of the cell. The germ-cell has its own particular type of physiological economy, and any variation in this type must conform in the first place to the necessities of germinal equilibrium. Whether such a variation is initiated by external or internal factors, it must be conceived primarily as a co-ordinated readjustment of these various reactions, and not as a mechanical rearrangement of some structural complex. That such spontaneous changes are possible may be presumed from an analogy with the similar readjustments that frequently occur in somatic life. Germinal variations must indeed be regarded as a kind of functional adaptation in the germinal cycle[2].

A successful variation must however not only conform to the necessities of germinal equilibrium, it must also result in a stable and efficient soma. We have already discussed some of the proximate factors in somatic equilibrium, which are extremely specialised and intricately co-ordinated in many of the higher organisms. Many of them are so elaborate that a serious modification of any single factor (for example the chemical composition of the duodenal hormones, the function of the erythrocytes, or the character of the neural linkages that form the presumptive bases of the primary instincts) would require a profound alteration in the whole economy of the organism if equilibrium were to be maintained.

If, therefore, as the mutationists suppose, the germinal and somatic cycles are functionally independent, is there any justification for assuming that a germinal readjustment conditioned only by the equilibrium requirements of the germinal cycle would also conform to the necessities of somatic equilibrium? There is no reason for such an assumption, for the types of physiological economy which

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[1] Including of course the meiotic divisions which usually occur at or near the end of the germinal cycle except in the case of haploid plants.

[2] This view is quite consistent with the discontinuous character of somatic morphological changes, and we believe not inconsistent with the quantitative aspect of these phenomena revealed by the experimental study of bisexual inheritance.

characterise the germ-cell and the soma are manifestly quite different. The postulation of parallel degrees of stability in germinal and somatic morphological characters is of no value whatever in this connection. What is implied by the theory of spontaneous variation is that those physiological reactions which are the most important factors in the equilibrium of the germ-cell, and therefore presumably the most stable, are always correlated with the most important and stable somatic structures and functions; or, to put it in a different way, that any changes in the germinal physiological reaction-complex which are mutually adjusted with respect to the functioning of the cell, and which affect the more important somatic characters, will do so in a way that is also mutually compensative with respect to somatic function. If the germinal and somatic cycles are functionally independent the probability of this happening in any single instance is very small; that such a parallelism would be maintained through a long succession of spontaneous germinal variations is too remote to be worth consideration.

A single unfavourable mutation, if sufficiently large, would at once give rise to a non-viable type, and if a truly specific and not an accidental individual phenomenon, would lead to the ultimate extinction of the species. The same result would occur sooner or later through an unfavourable combination of smaller mutations or a series of minute harmful changes in the same direction. The advocates of orthogenesis have indeed frankly admitted such a possibility, as for instance Duerden's suggestion that there is a progressive diminution in the toes of the African ostrich, which will lead to its ultimate extinction. A similar progressive change in a more vital organ, such as the cardiac valves, unless correlated with other compensative variations in the structure of the heart and the circulatory system, would attain the same result much more speedily. But, as we have seen, if germinal changes are spontaneous, there is no reason why they should be mutually compensative in relation to somatic function. There is, of course, the possibility that the soma itself is capable of effecting some readjustment during ontogeny; but this must necessarily be very limited or otherwise the facts of heredity would be meaningless.

We have endeavoured to show in the above discussion that the continued hereditary transmission of the physiologically important structures and functions through long phylogenies implies that there is some kind of functional interaction between the germinal and somatic cycles as a result of which germinal changes, at least such

as affect these characters, are limited and co-ordinated in relation to the physiological necessities of somatic equilibrium. Variation must therefore, in this respect, be regarded as a function not of the germ-cell, but of the whole organism.

The above argument refers primarily to determinate specific variations and is admittedly not so cogent in the case of sporadic individual mutations; for if the latter were small and fortuitous there may be sufficient latitude for readjustment during ontogeny, and the unfavourable combinations would be eliminated by individual extinction. But fortuitous variations, whether continuous or discontinuous, will not account for the evolution of complex functional mechanisms which involve the co-ordinated interaction of several different structural characters without the arbitrary postulation of various correlations that virtually deprive them of their fortuitous character. As we shall subsequently see, such sporadic mutations unquestionably do occur, but they are not very important factors in evolution.

IV. If, therefore, germinal variations are in some way somatically conditioned, it should be possible to learn something of the nature of this conditioning process. The Lamarckian theory of functional inheritance is an attempt in this direction, but its application is obviously restricted to heritable characters for which it is possible to assume an antecedent somatic origin. We shall endeavour to show however, that there is a more general relation between the somatic and germinal cycles, of which alleged instances of functional inheritance are only special cases.

It has been previously noted that different hereditary types are not all equally efficient from the physiological point of view. Let us proceed therefore to examine if germinal variations are in any way correlated with physiological efficiency. Now efficiency can be defined most exactly in terms of *energy*. The organism is continuously or periodically assimilating energy from the primary environment. Some of this energy may be conserved for a while in the secondary environment, *e.g.* as the internal reserve food material of plants, as the energy of the nervous system available for complex psychical activities, or as the external food stores accumulated by the instinctive and intelligent behaviour of the higher animals. Eventually, however, it is all dissipated in the various environmental reactions associated with the different organic activities. The relative efficiency of different types is the *ratio* of the energy assimilated to that expended in the process of assimilation. This definition is applicable to all

cases from the simplest unicellular plants and animals to the most complex associations of different individuals.

As a practical criterion however, this standard is of very little value, as the way in which the surplus energy is expended varies enormously in different types. It is necessary therefore, to have recourse to more proximate tests and to confine comparisons within certain restricted limits in which the type is relatively constant. Particular groups of structures and functions that are physiologically important and have a long phylogenetic history are most convenient for this purpose.

Now this test can be applied in two ways—by an indirect comparison of general types in any presumptive line of phylogenetic descent, or by attempting to ascertain directly the effect of observed mutations on somatic function. So far as the first method is concerned, there is the obvious difficulty that we have nowhere any exact knowledge of the phylogeny of existing species, and so must be dependent for the most part upon the comparison of different collateral types. Paleontological data are of value only in comparing anatomical characters, the functions of which are known in living forms. Within the limits we have stated, there is, however, marked evidence of a progressive increase in physiological efficiency in the course of phylogenetic development, as may be illustrated by the evolution of: (1) the photo-assimilatory apparatus of green plants; (2) the vascular system of pteridophytes; (3) the circulatory and respiratory system of vertebrates; (4) the nervous system of mammals; (5) the social organisation of hymenopterous insects.

The progressive increase in the efficiency of these physiologically important characters implies, even more than their observed stability, that germinal variations affecting them are mutually compensative with respect to somatic function. This may arise as the result of large mutations affecting several characters being internally compensated, or as the result of parallel smaller mutations being gradually compensated with respect to average somatic conditions.

When however we proceed to examine the various kinds of discontinuous variations of which we have actual experience, there is very little evidence that they generally have any favourable effect upon the physiological efficiency of the organism, or that they are in any way mutually compensative. On the other hand, the great majority of observed mutations, especially those which occur under experimental conditions, are confined to *single physiologically unimportant characters*. Thus among plants we not infrequently observe

small mutations which affect: the colour of the petals; the number of petals, sepals and bracts; the presence or absence of external hairs on leaves and stem, of awns on the glumes of grasses; the shape of the leaf; the amount of anthocyanin pigment present; and many presumptive wild mutations of a similar kind which have been held to account for varietal differences. In animals may be mentioned mutations which affect: the colour of the hair and the colour-pattern; the colour and shape of the feathers in birds; the shape of the comb in domestic fowls; the size and proportion of the skeleton; the presence or absence of horns in cattle; eye and body colour in Drosophila; the colour and size of the shells of gasteropods, and the occasional appearance of sinistral varieties in normally dextral forms; the artificially induced mutations in the colour markings of Chrysomelid beetles observed by Tower. There are many presumptive mutations of the same kind, such as the enormous variety in the spicules of sponges and in the tests of Radiolaria. In some cases, variations of this class may affect several different superficial characters simultaneously.

In the case of domesticated plants and animals indeed, the physiologically less important characters become apparently plastic and may vary enormously in even the same species. On the other hand, those structures and functions which are important proximate factors in somatic equilibrium, such as the mechanism of the nervous, circulatory and digestive systems, generally have a long phylogenetic history, and by no process of artificial selection can they be changed appreciably[1].

There do, however, occasionally occur marked single mutations in the stable and physiologically important characters and these are nearly always harmful, if not actually non-viable or incapable of hereditary transmission. In plants may be mentioned: sterile anthers; petaloidy in flowers; the absence of chlorophyll; also many of the Œnothera mutations which are associated with changes in the number of chromosomes. In animals: inherited sterility; albinism; the absence of the coagulating function of the blood; congenital structural defects in the eye, the heart, the thyroid gland; brachydactyly and other inherited deformities; congenital mental diseases.

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[1] *I.e.* rendered *more* efficient or changed in type. *Pathological* variations of the kind next considered may of course be selected. There is also the possibility that the prolonged exposure of a species to domesticated conditions by wholly altering the conditions of energy-assimilation (like parasitism) may itself result in a new position of somatic equilibrium in the manner subsequently discussed.

It is probable that we are really dealing here with three distinct classes of phenomena:

(*a*) Sporadic *individual* mutations affecting the less important characters. These are not apparently related in any way to the efficiency of organic functions, and must be regarded as more or less autonomous hereditary changes in subordinate physiological processes that are not important factors in somatic equilibrium. They probably account for the majority of the differences between domesticated varieties.

(*b*) Truly specific mutations. These also affect only the physiologically less important characters, but they are determinate and occur sooner or later in all members of the species, if conditions remain unchanged. They are often co-ordinated and may affect several characters simultaneously, but they may be individually not distinguishable from the first class. Variations of this type are mainly responsible for the observed morphological differentiation of natural species, and their origin will be discussed later.

(*c*) Sporadic *individual* variations affecting the physiologically important characters and which are always harmful. These are sudden changes in stable structures and functions that have a long phylogenetic history, such as the mechanism of vision, or the functions of the blood. They are essentially abnormal phenomena equivalent to fœtal monstrosities in somatic development.

Now so far as the *individual* mutations are concerned, they will be subject to the operation of natural selection in the Darwinian sense. It is probable that many of the superficial specific adaptations to special environmental conditions, such as protective coloration and complicated floral mechanisms in relation to insect pollination, have arisen in this way.

On the other hand, the second class of variations which are truly specific, and not fortuitous, will be incapable of individual selection, and will presumably occur sooner or later in all members of the species if environmental conditions remain unchanged. But as we have seen that observed mutations of this type do not generally affect the physiologically important characters, we have still to determine the type of variation which is responsible for the progressive increase in the efficiency of organic functions in the course of evolution. The probability is, that this is due to similar specific mutations, each so minute as to be non-recognisable individually, but cumulative and mutually compensative. There is no evidence

whatever of large mutations that give rise at a single step to new and efficient types of physiological economy.

What therefore is the relation between these two distinct classes of specific variations? It is suggested that the relatively large and discontinuous changes in superficial characters may have little or no evolutionary significance in themselves, but that they are correlated with the minute progressive changes in the more stable organic functions. As an illustration, may be mentioned the effects of slight changes in the amount or composition of the internal glandular secretions upon the morphological structure of animals, a factor which Keith considers to have been very important in the differentiation of the human species. It is possible of course that such correlated variations may, in some cases, be themselves of the orthogenetic type.

To recapitulate, there are four main classes of heritable variation:

(1) Minute cumulative changes in the more stable organic functions which are mutually compensating and tend generally to increase the efficiency of the organism, as an energy-system.

(2) Correlated with the former are variations in the physiologically less important characters, often relatively large and discontinuous, which account chiefly for morphological differentiation in allied species and genera.

(3) Sporadic individual mutations affecting unimportant characters. These may be selected and so give rise to specific adaptations and domestic varieties.

(4) Abnormal sporadic variations in important and phylogenetically stable characters which are always harmful and generally eliminated by natural selection.

Variations of the first class must be regarded as by far the most important factor in evolution, since they set the physiological type and maintain its stability. They play the same rôle in phylogeny that functional adaptation plays in a single life. The primary environment is in both cases very important, but whereas functional adaptations are compensatory readjustments with respect to ephemeral changes in external conditions, the trend of germinal variation can be influenced only by changes in *average* conditions that persist for a considerable period and so permanently modify the conditioning factors in organic equilibrium. The rate at which such specific readjustments can be effected must, however, be regarded as a property of the type; consequently when external conditions change too rapidly, a phylogenetic readjustment may become im-

possible and extinction will follow. There are abundant paleonto-
logical examples of this phenomenon, the general hiatus in many
phyla at the end of the Cretaceous period being the most marked[1].

It may sometimes happen that a trend of variation conditioned
by some persistent environmental change results in an effect mor-
phologically identical with that produced by functional adaptation
in the same circumstances, as for instance in some of the ecological
adaptations of desert plants. But in general the two processes must
be regarded as analogous rather than interdependent or identical.
In any case, it is only where functional changes affect the major
factors in somatic equilibrium that they can produce results morpho-
logically identical with those due to the more fundamental germinal
variations. The Lamarckian theory on the other hand is generally
used indiscriminately to account for the origin of even such superficial
characters as callosities on the skin, *i.e.* precisely those where
sporadic mutation is the most prevalent[2].

As however we have seen that the higher types of functional
adaptation involve compensatory readjustments which are apparently
independent of environmental changes, so the increase in efficiency
in phylogenetic evolution must be regarded as an intrinsic property
of the organism, and the environment as only a limiting factor.

V. We must now consider if it is possible to discover any general
biological interpretation of these facts. It is suggested that they
can be satisfactorily and consistently explained in terms of the
activity-theory of the organism discussed at the beginning of this
paper. From this point of view the succession of generations con-
stitutes a periodic sequence of events, in which there are recognisable
recurrent phases. The sequence is not however a simple one, and can
be resolved into a number of associated subordinate series that we
have termed activities. The germinal and somatic cycles are two
primary associated activity-systems.

It is, as we have seen, characteristic of the biological individual
that its constituent activities are functionally integrated. This
phenomenon of integration applies to the relation between the
germinal and somatic cycles, no less than to the various activities
which take part in somatic development. The way in which germinal

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[1] A change in average conditions for any particular species may of course
also result from the development of a particular character in some other
species.

[2] The *indirect* evidence in favour of functional inheritance, such as the
phenomena of Recapitulation, is equally consistent with the view discussed
here.

variations are *conditioned* by the course of events in the somatic cycle must be referred to this integration of activities. We have seen that even so far as normal ontogeny is concerned, it is not possible at present to give any interpretation of the mechanism of functional integration in either physiological or 'psychological terms that is applicable to all cases. It must simply be accepted as a postulate of empirical biology that describes most satisfactorily the manner in which environmental changes are observed to be constantly correlated in the development of different types of organism. In the same way we are not concerned with the physiological or other mechanism of the functional interaction between the germinal and somatic cycles. The organism is a unity of which they are both integral parts.

We have seen however, that in the reactions between different individuals there are various degrees of functional co-ordination, as for instance in the different types of symbiosis previously discussed. So also in the same organism the various activities are not all integrated in an equal degree. To use a modification of the previous simile, we may compare an individual life to a twisted cord that has been partially unravelled leaving a central intact bundle of strands surrounded by detached fibres and broken fragments. The central strands represent the nucleus of highly-integrated activities, which incorporate those reactions associated with the more stable structures and functions; the detached fibres are marginal activities corresponding to subordinate physiological processes; while the broken fragments represent sporadic and ephemeral reactions, which, while forming part of the whole reaction-complex at any moment (the cross-section of the cord), are not co-ordinated in any definite sequence in the course of development.

Variation must be regarded from this point of view as a periodic change in the normal sequence of events. It may originate in some new environmental factor, or it may not; but in any case the continued stability of the organism is dependent upon the persistent co-ordination of the subordinate activities throughout ontogeny. Hence variations affecting activities that are highly integrated will be co-ordinated and their corresponding reactions mutually compensative; where the activities affected are marginal, variations will be more autonomous and the corresponding reaction-changes apparently fortuitous. Sporadic and ephemeral reactions which are quite uncoordinated may vary indefinitely without being accompanied by any compensative readjustments in either individual or racial

development. Finally, there are occasional cases of functional dissociation of the more stable activity-systems that may give rise to abnormal and retrogressive types.

The process of phylogenetic evolution is not, however, analogous to an unravelling of the strands of a cord, but proceeds in the reverse direction. In the succession of generations the organism is constantly experiencing new reactions that are not a normal feature of somatic development. These may have an obvious external origin, such as many of the accidental and inconstant bodily movements of plants, or they may arise as abnormal fluctuations in internal physiological processes. In the higher types, fortuitous muscular movements of the various organs are also included. Our theory implies that these sporadic and ephemeral reactions become progressively co-ordinated into definite sequences or activities, which in turn become integrated with the more focal activity-systems. This co-ordination does not however take place fortuitously, but as a definite selective synthesis conditioned at each step by the physical limitations of the environmental factors involved[1].

Let us consider in illustration the evolution of a complex type of instinctive behaviour, such as that of various species of solitary wasp, which capture caterpillars, paralyse them by a sting in the cerebral ganglion, and then immure them in their mud cells as a source of food material for their own larvae, when the latter have hatched from the egg. We shall assume to begin with that the insect has already acquired a capacity for co-ordinated bodily movements of various kinds, and that in the course of its life such movements constantly occur in relation to a variety of external circumstances[2]. When the instinct has been perfected, a particular group of bodily movements has become co-ordinated in a definite sequence in relation to a particular set of conditions. The question we have to consider is how this co-ordination has been achieved. From the Darwinian standpoint chance movements are presumed to become co-ordinated in relation to chance stimuli in any direction (as a result of fortuitous inherited linkages in the nervous system), and the final combination is attained by the elimination of less efficient combinations through differential individual extinction in the struggle for existence. The

[1] This is of course a proximate interpretation consistent with the method of analysis we have adopted; it does not however preclude the possibility of an ultimate physiological interpretation of the same facts if they can be analysed completely in a different way.

[2] This implies of course that a complex type of behaviour has been already evolved.

mutationist view, at least in its crudest form, would postulate that
a specific germinal variation or a succession of such variations hap-
pened to give rise to the neural linkages requisite for the perfected
instinct. The Lamarckian interpretation on the other hand, if applied
to this case, would assume that the co-ordination was first perfected
by trial and error during somatic life and the efficient combination
then gradually stabilised in inheritance. This however would imply
the pre-existence of a type of functional adaptation (intelligent
behaviour) of which there is no evidence at this stage of evolution.

   The interpretation we adopt when expressed in ordinary biological
terms is essentially a combination of the mutationist and Lamarckian
theories. We postulate a selective synthesis from sporadic bodily
movements which is neither germinal nor somatic, but *organismal*
in character. The stable and efficient organic function in question
has resulted from a series of steps which, while truly specific and
appearing first as inherited variations, yet represent each a definite
reaction on the part of the whole organism in relation to a particular
set of environmental conditions. According to this view, if the suc-
cession of generations in the phylogeny of any particular species of
wasp could be telescoped in the manner of a cinematograph film, we
should simply observe a progressive perfection of the mechanism of
muscular co-ordination somewhat analogous to that observed in the
acquisition of a complex habit. The particular bodily movements
which have been selected and synthesised in this way constitute a
new organic activity. In its earlier stages the new type of behaviour
will vary sporadically, but as it becomes progressively integrated
with the more focal activity-systems it will become more stable. On
the other hand, those bodily movements which have not been co-
ordinated into definite sequences may still take place, but they will
remain fortuitous and indefinitely variable[1].

   There are, however, in different species of wasp considerable
modifications of this particular type of instinct relative to the kind
of prey, the method of capture, and the manner in which it is dragged
to the nest of the insect, which clearly represent divergent trends of
racial evolution. But there are reasons, based upon a comparative
study of different species and other grounds, for believing that these
various specialised types of instinct were all preceded phylogenetically

   [1] This example has been chosen for simplicity because the phenomena are
largely external and therefore more capable of observation. The internal
bodily characters of plants and animals we presume to be evolved in the same
way, the only difference being that the sporadic reactions which become
co-ordinated are of another kind.

by a more generalised type in which the ova were deposited indiscriminately in the bodies of many different kinds of prey, and that no attempt at concealment was originally made. We may further presume that the process of immuring the captured larva or other animal developed next in order, and that the various methods of effecting this operation are of subsequent growth. To understand the origin and significance of these specific divergences, each of which presumably implies a progressive increase in efficiency in its own particular phylogeny, we must again refer to the effect of environmental limiting factors on organic equilibrium. In this particular case the limiting factors are mainly the anatomical structure of the insect previously evolved, and the character of the prey with which it deals. For a particular type of insect and a particular kind of prey there are, we assume, only a restricted number of different types of co-ordinated muscular actions by which the operation in question can be efficiently performed. These different systems may not be equally efficient, but each is complete in itself and their constituent elements are not mutually interchangeable. There is probably, however, some major determining factor, such as whether the prey is pushed before or dragged after the insect, which sets the type in each case, and which becomes established at an early stage in the evolution of the instinct. In other words, once a particular group of bodily movements have become co-ordinated to a certain degree (and therefore acquired a certain phylogenetic stability), the line along which a further increase in the efficiency of the process is possible is more or less physically determined. But the actual increased efficiency along this line is still of course an intrinsic property of the organism. All the specialised specific types originated in a generalised type where the movements were much more diverse and sporadic, and the factors which determined in the first instance what particular group became the nucleus of the new line of development, may have been some chance difference in local conditions[1].

This principle of environmental limiting factors is a very important one and is applicable to the whole field of phylogenetic evolution. A particular type of physiological economy becomes more efficient up to a certain point as the result of a succession of co-ordinated germinal changes, but a condition may be reached eventually in which little further improvement is possible on the same lines, and

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[1] We do not preclude the possibility that some of the minor details of the various instinctive types may have arisen by the effect of natural selection upon sporadic variations in characters *that have not yet become stabilised.*

the only subsequent variations will be sporadic and adaptational. The continued existence of representatives of archaic forms, such as unicellular algae and protozoa, liverworts, mosses, sponges, corals, etc., must be ascribed to this fact. It is important to note that from our point of view the relative fixity of these forms, which may be compared to truncated lateral branches in the evolutionary tree, is not due to any peculiar quality of the germ-plasm, but is an intrinsic physical limitation imposed by the type of physiological economy they have developed. A marked further increase in efficiency could only result from some fundamental change in organisation, which is inhibited by the essential stability of the more important structures and functions. They may however still undergo considerable changes in relation to environmental conditions, while retaining the same general type. The divergent trends of variation that have led to the more efficient types must therefore have been initiated in less specialised forms. The inferior stable types are those which failed to take the right turning at some critical period in their phylogenetic history, when a deflection of the energy of the organism into alternative channels was possible.

From the biological standpoint, the evolution of the higher animals is characterised, as we have seen, by the synthesis of sporadic and ephemeral reactions with the primary environment into complex activities, which in turn become further integrated with the more stable activity-system of the organism, subject of course at each step to the limitations imposed by the environmental factors involved. In the higher types a modification of instinctive or intelligent behaviour is one of the most powerful means of increasing physiological efficiency, and it also permits the further integration of the whole activity-systems of different individuals into the still more efficient social groups.

From the energy standpoint, this process of progressive integration is represented by an increasing complexity in the secondary environment of the organism. In plants the energy-reactions which are co-ordinated in somatic development are practically all internal or physiological in character. The higher animal types on the other hand have acquired a capacity, as it were, for reaching out into the primary environment far beyond the limits of their morphological bodies, and progressively incorporating diverse external reactions within the sphere of their economic system. This "external physiology" is very varied. In its simplest form it is represented by various supplementary mechanical devices, such as cells, nests, etc.,

usually associated with the self-preservative or reproductive instincts. At a higher stage it includes the complex external economic machinery of insect colonies which often incorporates within itself the physiological reactions of different species of organism. On a different line of evolution is the progressively increased capacity for functional adaptation during somatic life which begins with the power of external movement, thus making the organism less dependent upon the primary environment, and finds its highest expression in intelligent behaviour.

All these various examples are essentially so many different ways in which simple types of organism have been rendered more efficient, either by an increased power of selection with respect to their reactions with the outside world, or by the superaddition of some external economic machinery to the internal physiological mechanism previously evolved.

MERISTEMATIC TISSUES AND PROTEIN ISO-ELECTRIC POINTS

By W. H. PEARSALL and J. H. PRIESTLEY

INTRODUCTION

I N some recent studies of the development of cork within the plant an attempt has been made to give a causal interpretation of the appearance of this tissue. There is one aspect of the whole problem which is neglected in both these communications, but which is really fundamental in dealing with the development of meristematic tissues. This aspect it is desired to consider in the present note.

Cork usually arises in fully differentiated and vacuolated parenchymatous tissue as the result of the appearance of a meristematic phellogen. Both this meristem and the cells formed from it are often completely filled with protoplasm and always much less highly vacuolated than most tissue cells. The transformation of tissues from a vacuolated, non-dividing condition into non-vacuolated, or only slightly vacuolated meristematic cells is characteristic not only of phellogen formation but also of the extension of vascular cambium across the ground tissue bordering the vascular bundles. In either

case the first sign of meristematic activity—as seen under the microscope—is that aggregates of protoplasm appear in the originally vacuolated cells, and from these aggregates new non-vacuolated or only slightly vacuolated cells are cut off.

Cylinders of meristem such as the vascular cambium and the cork phellogen are characteristic features of Dicotyledon anatomy, and in order to understand the causal machinery concerned in their production it would seem essential to know how the original dense aggregation of protoplasm in the vacuolated cell is brought about, and to determine the conditions necessary for its maintenance. The phenomenon recalls the precipitation of protein from a sol, or the contraction of a protein gel with loss of water. These are processes upon which a considerable amount of light has been thrown by recent investigations in physical chemistry. The object of this note is to show that the appearance of these meristems seems to be correlated with a gradient of hydrogen-ion concentration in the tissues in which they arise and that in the light of recent physico-chemical investigation this fact may have very great significance.

The Gradient of Hydrogen-ion Concentration across a Cambial Zone

Many writers have observed (see Atkins (1)) that it is now possible to obtain a rough idea of the reaction (hydrogen-ion concentration) of plant tissues by employing the modern range of indicators (5) in contact with sections of fresh material. When the tissues near an active cork cambium are examined in this way, the newly-formed cork cells are found to be extremely acid in reaction, approximately pH 3·0, this being due apparently to the fatty acids liberated from the rapidly differentiating cork cells (Priestley and Woffenden (9)). On the other hand, within the phellogen, the cortical layers of paren-chyma have normally a reaction between pH 5·5 and 6·5. Hence the cambial layer lies across a fairly steep gradient of hydrogen-ion concentration.

In the case of the cambium of normal vascular tissues the con-dition is somewhat different. Atkins has recently stated (1) and we have frequently observed, that the reaction of the xylem is relatively acid (pH 4·3 to 5·0), whilst, as Sachs pointed out many years ago (11), the phloem is relatively the most alkaline tissue in the plant and may frequently be alkaline to litmus (pH 7·8 or more). It is therefore a striking fact, and one worthy of much further investigation, that each of the two cambial meristems of the normal Dicotyledon lies

across a marked gradient of hydrogen-ion concentration, but that the gradients run in opposite directions and differ markedly in range. Any attempt to elucidate the significance of this fact necessitates some discussion of recent work upon the colloid chemistry of the proteins.

THE ISO-ELECTRIC POINT OF A PROTEIN

Colloidal systems dispersed in water frequently bear an electric charge and, as a result, if an electric current is passed through the solution, the charged particles migrate slowly to the oppositely charged electrode, a phenomenon known as electro-cataphoresis (see Burton (4)). It has long been known that the electric charge upon the dispersed particles varies with the reaction of the dispersal medium, and also that there is one reaction of the medium at which the colloid will behave as if uncharged. This reaction Hardy (6) has termed the iso-electric point. Loeb (7) has developed further the significance of this fluctuating electric charge in certain types of colloids, and as a result of his work it is evident that for proteins, at least, the position of the iso-electric point is one of the most significant properties of the pure substance. Proteins may behave either as acids or as bases, in virtue of the carboxyl (—COOH) and amino (NH$_2$—) linkages of their constituent amino-acids. Loeb has shown that if a pure protein is dispersed in a medium containing electrolytes, which is more acid than its iso-electric point, then the protein bears a positive charge, behaves as a base and forms in part a protein-acid salt. On the other hand, in a medium more alkaline than its iso-electric point, the protein is negatively charged, behaves as an acid radicle and forms salts of the base-proteinate type. With this key to their behaviour, Loeb has shown convincingly that the complex molecules of the proteins combine according to the ordinary stoichiometric laws of classical chemistry. A very confused and perplexing field of colloid chemistry has thus been reduced to something like order. At the moment, however, we are only concerned with one of the very wide range of phenomena that have been elucidated. At the iso-electric point some of the physical properties of a protein are at a maximum, others at a minimum. Thus at this point proteins show most tendency to precipitate and a minimal tendency to swell in water (Loeb (7) and literature cited therein). In other words if a protein gel or the solid phase of a protein sol be under consideration, with changing reaction of the medium there will be a change in the distribution of the water within the protein,

and at its iso-electric point the protein will most readily part with water if in contact with other systems which tend to absorb water.

Now in the case of a vacuolated cell the cytoplasm of the cell may be considered to retain water as a result of the swelling properties of its proteins. Suppose we take the case of a series of such cells lying along a gradient of hydrogen-ion concentration.

If somewhere along the gradient lies the iso-electric point of one of the main constituent cell proteins, then at this point there will be a distinct tendency for the cytoplasm to lose water to cells on either side, which are at different hydrogen-ion concentrations and therefore have greater affinity for water. Under these circumstances, the gradual accumulation within such cells of a dense, unvacuolated, or only slightly vacuolated, mass of protoplasm whose water is lost to neighbouring cells is at least conceivable. Furthermore, such a tendency to water loss would produce a definite tendency towards synthesis, rather than towards hydrolysis. The characteristic metabolic processes in plants are condensations in one direction, involving the elimination of water, and hydrolyses in the other direction, involving the addition of water. We may, for example, visualise the particular case of protein synthesis as being essentially the union of two amino-acids or their complex derivatives, A and B, to form the more complex substance, C, and water, e.g.

$$A + B \rightleftharpoons C + H_2O.$$
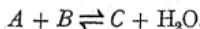
Now according to the law of mass action, the concentrations of these four substances are proportional to one another (cf. Bayliss, 2, p. 239) as follows:

$$\frac{(\text{conc. } C)\ (\text{conc. } H_2O)}{(\text{conc. } A)\ (\text{conc. } B)} = \text{a constant.}$$

From this general equation it is clear that in order to increase the concentration of C the concentration of water must be reduced. In other words, starting from a given point, elimination of water will lead to synthesis of C, addition of water will lead to hydrolysis of C, until a new equilibrium is attained. From this point of view elimination of water appears to be an essential condition for synthesis in plant meristems.

Our test-tube knowledge of protein synthesis enables us only to envisage such condensation by analogy, but the extensive series of carbohydrate syntheses and of carbohydrate ester syntheses by the use of enzymes in alcoholic media with low percentages of water (Bourquelot and Bridel (3)) supply strong evidence in support of

such an argument. The idea that a plant meristem requires to be relatively free from water in order to persist in its synthetic metabolism has already been employed in reference to the apical meristem (Priestley and Tupper-Carey (8)) and here also it is now suggested that ultimately the growing protoplasm will be found to maintain a certain equilibrium reaction favouring the withdrawal of water by the osmotic action of the vacuolated cells bordering upon the meristem.

A gelatin gel at its iso-electric point still retains a considerable amount of water by a mechanism which is far from clear and which may still be vaguely expressed under the term imbibition. Many proteins, however, precipitate at their iso-electric points and can be removed from solution as amorphous solids, retaining no more water than any other wet precipitate and drying as readily. The natural assumption at the present time appears to be that the normal meristem with protein at its iso-electric point has little or no more affinity for water than such a precipitated protein and as fast as water is released in the system, by the condensation processes accompanying synthesis, it is lost to the surrounding tissues. The cambial meristem should therefore remain under conditions permitting synthesis and growth, so long as the hydrogen-ion gradient is maintained and the necessary food materials are supplied.

THE ISO-ELECTRIC POINTS OF PLANT PROTEINS

An essential condition for the production of meristematic activity on the above hypothesis is that the most important proteins in tissues capable of growth should have iso-electric points at reactions lying between pH 3 and 6. Few published data exist as yet upon this subject, but a variety of tissues and tissue proteins (from ten diverse genera) have been examined in this laboratory. The unpublished results justify the statement that in the cases investigated the iso-electric points of the principal proteins in plant tissues lie between the required limits, and are frequently about pH 4·4. There, are slight indications that the proteins of the tissues of aerial organs may differ in this respect from those found in subterranean organs.

CONCLUSION

While the ground traversed in this brief statement must be covered again, probably many times and over a much wider field, before the generalisation suggested can be accepted even as a working hypothesis in physiological anatomy, it is hoped that a case has

been made out for considering many well-known facts of plant anatomy from the following standpoint:

Synthetic metabolic processes are usually condensations involving the release of water from the reacting substances. As reversible reactions, such synthetic processes are therefore facilitated by the removal of water from an actively synthesizing (or growing) meristem. Hence the fact that the cambial meristems lie across gradients of hydrogen-ion concentration suggests that they occur at that point where important constituent proteins are at their iso-electric point. These proteins thus possess minimal affinity for water, which may, therefore, be withdrawn from the meristem to the osmotically active cells on either side. An examination of the iso-electric points of tissue proteins so far as these are known suggests that the majority of such proteins have iso-electric points lying within the range of hydrogen-ion concentration observed on the two sides of a cambial meristem.

These correlated data are at least suggestive and in the case of the cork phellogen no other causal mechanism has been suggested, so far as the writers are aware, which gives an adequate basis for the production of a new meristematic unit within an originally vacuolated cell. A moment's consideration will show that the same mechanism would account for the development of a cambium within the procambial strand and its gradual extension as interfascicular meristematic tissue across the primary medullary ray. The problem of the apical meristems has been left on one side in this paper as it presents additional difficulties in observation and interpretation. There seems to be little doubt that here also water is withdrawn from the meristem in a somewhat similar way, although the manner in which the meristem proteins are maintained at the iso-electric point is not at present clear.

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CONTENT, METHODS AND MEASUREMENTS IN THE TEACHING OF ELEMENTARY BOTANY

By FREDERIC E. CLEMENTS

THE first step in carrying the ecological method of teaching into effect is to ascertain what is actually being accomplished in a particular course. This demands the clear recognition of the objectives sought. The definite formulation of objectives in teaching is perhaps as rare as the use of experiment and measurement, and a large number of teachers have but a vague idea of what they are striving to attain. Probably the aim most frequently expressed is to give the student an adequate idea of the subject, but this requires closer definition. To some it means a mass of facts, while to others it signifies an emphasis of principles and a disregard of facts, but to nearly all it denotes the covering of the traditional ground in the traditional time. In biology the almost universal aim is to "teach evolution," with which is usually combined the desire to dignify the subject in the student's eyes and to stress the great names in its development. It seems superfluous to point out that these are professional objectives, having in mind the interests of the professor and his subject, and assuming that these are identical with those of the student. They explain the prevalence of lecture and text-book, and throw light upon the abyss between scientific men and the public. They are the inevitable outcome of extreme specialization and of the practice of using such specialists as teachers of general students.

In the sharpest contrast to these objectives are those that spring from making the interests and needs of the student paramount, and

the interests of professor and subject secondary. Foremost of all are developing the student's interests and providing him with the means of obtaining and testing his own facts and giving them meaning. Initiative and independence are indispensable to the teaching that places the student first, and they are far from undesirable for the professor and the subject. The spirit of inquiry, developed with sympathy and directed with understanding, is incomparably the greatest of all the results that can flow from teaching. Moreover, while it is clear why the humanities have failed to develop this spirit, it is incomprehensible that professors of science, themselves investigators, should have so nearly succeeded in extinguishing it. The spirit of inquiry alone can lead to right thinking that is intrinsic, and the latter is the sole guide to the objectivity that is as imperative in human affairs as it is in science. Inquiry, thinking, and the application of knowledge are but reciprocally interlocking parts of the basic mental process, and teaching based upon them no longer need concern itself about the relative values of training and information.

The objectives once determined, the next step is to discover whether they are being realised. To some teachers such scrutiny will appear as superfluous as it is undesirable. They possess the evidence furnished by examination paper and notebook, and questions as to the independence of the work and the permanence of the results are inconvenient. The student has drawn, even though he has not seen with his mind, what was shown him, and has written what was told him; the requirements are met and the credit granted. To the teacher with misgivings as to the student's real accomplishment, the unannounced examination will reveal the actual situation, though it must be a complete surprise and not the "unannounced" kind that regularly appears at a certain point in the course. This method is always revealing even when given a few weeks after a subject is completed or but a week or two after a set examination has discharged the student's responsibility. But it is most compelling a month or two after the course has closed, and conclusive at practically any time during the next year or two, particularly after the student has left the field altogether. In the latter case, such tests are difficult to apply, but they can be made, and they will be by all teachers who are as much interested to know the truth about their teaching as in their special fields of research. In this connection certain teachers will insist that they do not expect students to retain the facts of the course, or even the principles necessarily, but

that the important things in teaching are the intangible values. This takes teaching out of the realm of science into that of faith, and needs no further consideration. Unexpected examinations are especially helpful to the teacher who is impressed by the ease and rapidity with which ground is covered by the aid of lecture, or text-book, and demonstration. Ease and rapidity are not conditions that accompany vigorous mental growth, as can be demonstrated by the unexpected, or, better still, the delayed examination. The slighter the stimulus the less the response. With an organ so little used as the mind the stimuli must be strong and continuous or periodic if they are to produce permanent results. Repeated experiments have proved that lecture, text-book and demonstration do not produce stimuli of this quality.

Even unexpected examinations are not capable of furnishing evidence as to results other than those obtained by the memory, except where it is possible to state a problem on paper, as with a problem in geometry. Adequate tests of mental development can only be made by means of practical and applied examinations, and, best of all, by the assignment of observations and experiments in which all of the work is done by the student, from organizing his plans and obtaining materials to carrying the plans through success-fully and drawing the proper conclusions. It is obvious that this is the method of scientific research, and equally obvious that teaching carried on in this manner needs no set examinations or tests. Every task is a problem that demands the student's best efforts in organ-izing, observing, experimenting, thinking and correlating. No examination of the traditional sort that may be given can have a tithe of the value in his mental development that is exerted by a new problem that demands a mastery of the preceding one for its solution. In this fundamental respect the process-inquiry method is not merely identical with that of scientific research, but it also accurately reflects life itself with its constant pressure of problems. The similarity runs throughout, and nowhere is this similarity closer than in the fact that contacts, like blunders, must be personal and repeated to bring usable knowledge. However, while properly graduated series of problems bring their own tests of development and achievement, knowledge of the exact stages must be constantly available to both teacher and student. This involves the measure-ment of the learning processes, as discussed later.

The content of general courses in biological science has been criticized more frequently than the method, chiefly because its

shortcomings from the students' standpoint have been more obvious. Dependable opinions by either the conference or the questionnaire method are to be obtained with difficulty, if at all, by the teacher from his own students, but this can be readily done by a colleague. It has been found that the students will discuss matters of content and method with entire frankness and great intelligence, when they feel that their confidence will be respected. The conference has decided advantages over the questionnaire in that it permits refining the students' views, but it is time-consuming, and may be supplemented by the questionnaire when categorical answers to definite questions are desired. The use of conference and questionnaire for twenty years, aided by all too rare volunteer criticisms, has confirmed the impression that the traditional content of biological courses is entirely foreign to the students' normal interest and needs, in spite of the fictitious interest often engendered by the teacher's enthusiasm and the student's docility. This is readily checked by the opinions expressed when courses have been changed to meet the students' views, but it receives its strongest confirmation from the evidence afforded by the usual general courses. Measured by the number of students that it discourages from advanced work, as well as by the much larger number that it fails to attract, such a course is the chief factor in the present unsatisfactory status of biology. Its effect is far-reaching, for in forms variously diluted it finds its way into schools, and comes to represent the only kind of botany and zoology that the public can know. Its disastrous effect is seen in the general attitude of the "educated" public toward biology, as well as in the almost complete disappearance of the amateur within the last few decades. While the differentiation of agriculture, horticulture, and forestry from botany, and of medicine from zoology was in some measure inevitable, it might well have taken place within the parent subject had biologists been more sympathetic toward human needs and less myopic as to applied science. The ground thus lost can never be regained, but it is still possible for biology to render unique service and regain lost prestige by increasing its sympathy and understanding.

If the course in general botany is to reflect these qualities in a large degree, the teacher must turn to his students for understanding. He may do this gradually and indirectly, dropping this section and adding that as he determines student response, and perhaps approximating to the desired result in a decade or more. Or he may subscribe fully and unequivocally to the principle that the students' needs and

interests are paramount to all other considerations, and at once set about making the students partners in the course. This does not in the least mean abdicating the functions of the teacher, but on the contrary giving them full scope, since in return for his knowledge of what is possible and how to do it, he gains the student's view of what is most worth while and of the most interesting manner of attack. Moreover, once he approaches the matter sympathetically, his own experience is invaluable in refining the students' ideas and in helping to decide between various suggestions. To one who has run the whole gamut of experience from stereotyped courses in evolution and morphology in which there was not the slightest idea that the student's viewpoint was a thing to be considered, through those in which there was a growing feeling that his interests should be wrought into the course, to the final stage where the student's interests and needs are regarded as decisive, there seems no possibility of hesitation as to the proper method. Indeed, if the student is to be animated by the spirit of inquiry as the primary motive force in learning, his right to include the subject-matter of the course in his investigation cannot be ignored.

In practice, the task of determining the content is an intrinsic and regular part of the course. It not only occupies the first period of two or more hours, but as much of succeeding ones as is necessary to determine the students' contacts with the plant world, to organize these into a desirable sequence, and to give the students a clear view of the relation of the parts to the whole. While one may entertain natural doubts as to the student's ability to "play up" in a game of this sort, these will quickly disappear when he realizes that his opinions and interests are being taken seriously. Of the several methods of approach that have been tried the most direct and successful is the one in which each student is asked what he most wishes to know about plants. This is done with the utmost informality in order to encourage the students to volunteer their views, and to make it possible to pass quickly from question and answer to an animated discussion in which the teacher appears a participant rather than an evident director. As the views are expressed, they are written on the blackboard in order to have them clearly before the class when it turns to deciding which of these represent best the interests of the class as a group. From this discussion results a sequence of projects in the order of their interest or importance, in which the teacher's chief share is to see that one permits of a natural development into the next, and that there are no impossibilities,

such as might arise from the season. For the sake of perspective it is desirable to sketch the work for the entire year, or for a term at the least, but it is best to determine the details project by project, as this enables the student to bring increasing knowledge and insight to the task. Finally, it must be realized that the outline thus obtained is a guide and not a mould, and that it is to be changed or modified as the developing interests of the class demand. In this connection emphasis should perhaps be given to the fact that by interests are understood those motives that impel the student to work and think rather than to gratify a passing curiosity.

While each region or locality may have its peculiar contacts or interests, all the beginning classes handled in this manner have been practically unanimous as to the most important things to include in the course. The things that plants *do* have proved much more compelling than what they *are*, and the student thus finds form and structure invested with a meaning to be gained in no other way. It is impossible to work with processes in plants without growing them, and this leads inevitably to their uses, patently the two oldest and most basic human contacts with plants. It is quickly realized that plants can be handled more readily and certainly when they have names, and this furnishes a convincing reason for discovering the floral earmarks of families and learning to read the solution of the flower's problems of pollination and seed-production in its structure. It does not, however, constitute a warrant for the use of keys and manuals, which belong only in advanced classes. The contacts with the visible plant world prove so numerous that the microscope enters the course but exceptionally if at all, and only when it is needed to give reality, as in the case of pollen, for example. Furthermore, the students' contacts with cultivated plants have been and will be much more numerous than with native ones, and the material of the course will be selected accordingly. Moreover, it will be disclosed that students do not need to be "taught" evolution, but that this is something they will discover for themselves in their work with flowers, with an understanding and insight entirely foreign to the traditional account. Finally, the out-doors is the world of students as well as of plants, and interest and results are enhanced to the degree that the work is done in garden and field, or the substitute that winter demands, the plant-house.

Since the student organises his work and does it independently, the laboratory is the seat of the entire work of the course. But it is a new laboratory—one close to the primitive meaning of the word,

which consequently includes all those places in which plants are at work, and students can work beside them. Hence, it excludes to the highest possible degree all the traditional laboratories into which living plants are rarely brought or in which they lead a sickly existence. Such rooms are as undesirable for students of plant life as they are for plants themselves, and there is no excuse for tolerating them longer than absolutely necessary. The difficulty of keeping the students at work in an environment of living plants throughout the course arises almost wholly from the position of the academic year. When the latter consists of four quarters, elementary courses in botany should begin in the spring and end with the approach of winter. Where the usual academic calendar, beginning in the autumn, is maintained, the sole adequate solution is the plant-house. Such a solution has proved entirely feasible in countries with a long severe winter, and should be much simpler in those with mild winters.

Ideally, the plant-house should be a greenhouse so easily modified as to temperature and humidity as to be equally desirable for students and plants, but in practice well-constructed greenhouses have been found to serve very well. Where a greenhouse is available but laboratory space lacking, the addition of a roofed wing with adequate side-lighting has proved very satisfactory. The plant-house should be located in a well-planned garden, preferably with space available for individual student gardens, and as close to field and forest as possible. Wherever universities are situated in cities, the department of botany should be detached and located in the suburbs, always preferably on the experimental ground of the department of agriculture when there is one. While the plant-house should be provided with studies for the teachers, it has neither lecture-rooms nor class-rooms, as none are needed. All the work, organization and discussion as well as experiment, is done in the laboratory, whether it be plant-house, garden or field. Such a laboratory, once established, will prove to be equally valuable for advanced and graduate courses as well.

The basic method of training the student to do all of his own work and thinking applies as well to questions of material and detailed methods of observation and experiment as to that of subject-matter. Mastery can be gained only by following each project throughout with true understanding, and it is essential that the student should select and obtain his materials. This may be done by the individual or by the group, though a combination of the two

plans is usually the best. Even more important is the planning of observation and experiment. In the light of experience it seems little short of useless to give the student detailed directions for each experiment, and the laboratory manual appears to be downright harmful in depriving the student of the opportunity of organizing his own work. Here the preferred method is to allow each student to work out a plan independently, and then to take these up in the discussion to obtain the group judgment as to the most promising methods. Dissenting opinions, however, must always be respected and put to the test, in order to lead the students to recognize that opinions, majority or otherwise, can be separated into wheat and chaff only by experiment and measurement. The execution of observation and experiment is an individual matter, but there are projects in which it is desirable and sometimes necessary to organize the students in groups. The responsibility of the student does not end with the completion of the experiment. He must first dismantle his apparatus, and return material and supplies to their proper places in condition for immediate use. Much more important, he must turn to the organization and interpretation of his results as the most essential feature of the entire project.

This is done in such a way as to give the memory and the power of thinking the maximum opportunity for development. No notebook is kept, though tables of readings and similar data are permitted. The teacher who requires that everything be entered in the lecture and laboratory notebooks will be highly sceptical as to this, but, if he subscribe to the statement that the relative values can be determined only by experiment, it is exceedingly probable that he will ultimately dispense with both. At any rate, no class so far studied has failed to prove that notebooks of all sorts almost completely inhibit the proper functioning of the memory and thinking processes. It has been found that the repeated handling of the results by the individual and by the group, and their constant use in succeeding projects and discussions renders them permanently available, the ability of the mind to respond to such conditions being quite beyond the belief of one who employs traditional methods.

While the student must feel individually responsible for his results and for giving them meaning, complete values can be obtained only through the group discussion. This is essentially an exchange of experiences and interpretations made with entire informality, but so guided by the teacher that actual progress is made and definite

decisions reached, though the latter may often be to the effect that more experiments or observations are needed. The group discussion entirely replaces lectures, and makes formal examinations unnecessary. It is held in the laboratory, whether plant-house, garden or field, whenever the progress of the work demands, and it profits greatly by the fact that materials and experiments are at hand for refining or checking statements and conclusions.

While differences in setting, equipment, seasons and available teaching periods make it impossible to give a detailed procedure that will be generally applicable, the general plan of organizing and conducting the work will be similar for all. The division of a class into sections is based upon the conviction that the sympathy and understanding that should exist between the teacher and each one of his students are impossible in a section of more than 25 and can be most readily secured in one of 20 students. Everything considered, the best arrangement of time consists of three 2-hour periods a week, provided this permits a half day for field work whenever needed. Under certain conditions two 3-hour periods will prove more convenient, but must be supplemented by visits to look after plants, make readings, etc. Since the teacher has no lecture or laboratory notebooks and no examination papers to burden him, he can give his best efforts to as many as three sections and still have half his time for investigation, a large part of which should be devoted to teaching methods and results. An elementary class of 500 students would thus require seven or eight teachers, which seems not at all excessive in view of the quality of the results obtained.

As has been already indicated, the first meeting of each section is given to the group discussion of the existing contacts of the students with plant processes and materials and to selecting those contacts that promise the greatest interest and values. In practically all cases this has resulted in placing first the behaviour of plant and flower, followed closely by the uses that man makes of plants. Identification and herbarium-making of the traditional sort receive little sympathy, and are replaced by an understanding of evolution and relationship gained through the first-hand study of the life-history and pollination of flower types. In the spring the choice naturally inclines to germination and the growth of the seedling, in the autumn to the life-history and pollination of the flower. In either event it is overwhelmingly in favour of function and it turns to form and structure only as the class discovers that these are

inevitable results of the former and in their turn serve to explain it. The decision as to the first work to be taken up is followed by a discussion of the general method of attack, which still leaves room for individual initiative as to the details. The actual work of observation or experiment is begun at the second meeting, a considerable range of choice being permitted as to material and method, while unity is secured by having all deal with the same project. The teacher's task is to stimulate and on occasion encourage, and to train the student to realize that he can secure nothing of value from the work except by his own effort. By holding the quicker students to greater accuracy and helping the slower ones to form simpler and more direct habits of thinking, the class will come through each project with a fairly equable preparation for refining their methods and interpreting their results by group discussion. The latter will often reveal the need for further investigation and point the way to the problem that should follow. Each problem must grow out of one or more of the preceding ones and be kept in the broadest possible contact with them, as it is only in this way that permanent and usable knowledge can be secured. Each student turns to his experience again and again for the materials on which he must build and the repeated use of them makes lecture, text, and notebook superfluous. No examinations are needed to test his memory or to help him "organize" his material, nor are they necessary for the teacher who is in constant touch with his individual students throughout the entire working period. Each problem is itself a test made under the eye of the teacher, and he requires in addition only occasional definite measurements of progress in the various learning processes, measurements which should be as intelligible to the student as to himself.

In experimental education, as in all research, knowledge rests upon quantities. Measurement is as essential to the student as to the teacher if either is to be sure that adequate development is being obtained and permanent results secured. Qualitative evidence of this is furnished by the inquiry method itself, since the ability of the student to organize and carry out projects with increasing demands is proof of constant growth. Such evidence renders examinations, both oral and written, quite unnecessary, except as they are used to measure improvement in memory. Quantitative results are necessary in the first place as an essential part of the investigation, but their great value lies in the certainty and accuracy with which the mental development of the student can be traced. Measurements make it

possible to determine in detail the response of the student to the content and methods of the course from the two different viewpoints, that of the teacher and of the student. Even better, they permit an analysis of the learning process in the case of each student, and hence afford the only basis for giving it complete and symmetric development. The student's part in measuring results is as great as that of the teacher, and constitutes an indispensable part of his training. The insight and objectivity that he thus develops with respect to his own deserts and accomplishment are beyond the belief of one who has taught only in the traditional way.

The measurements employed were first developed twenty years ago, before the appearance of the intelligence tests, and consequently differ from the latter in several respects. The most important of these is that they are analytical, and deal directly with what are conceived to be the essential steps in the learning process. As a consequence they are applicable to all subjects when these are taught by the process-inquiry method. In addition they are based upon the assumption that memorizing is not the sole or even the paramount objective in teaching, but that it is merely an intrinsic step in the learning process. Perhaps a unique feature in their application lies in the fact that they are used to measure progress rather than to determine capacity once for all. This is in conformity with the working hypothesis that all normal minds are capable of development to a degree altogether unexpected, when subjected to the proper stimuli and environment. For purposes of study and measurement the learning process is divided into six steps or sub-processes, termed observing, experimenting, remembering, reasoning, relating and applying. These permit of further analysis, especially in psychological terms, such as perception, attention, etc., but the sub-processes given were chosen because they are readily intelligible and conform to the learning process of the race as well as the research method of the scientist. It is obvious that learning as ordinarily understood may begin or end with almost any one of these, though remembering regularly obscures all the others. On the other hand, real learning, by which is meant complete learning, must begin with observation and end with application. In short, learning that is not applied to life, in terms of service, efficiency, comfort, or enjoyment is regarded as spurious.

The limits of a paper do not permit an adequate treatment of the measurement of the various sub-processes, but this may be exemplified in the case of observation. The students set themselves

the task of observing independently every possible point in an object, such as a flower or a shoot, or every step in a process such as pollination or a tropism. The group then determines the total number of points that should be observed, and this constitutes the norm in relation to which the performance of each student is reckoned, as a ratio or percentage. A similar object or process is employed to determine the time element, but this is treated as an indicator rather than an essential part of the norm, owing to the fact that, while remembering can be speeded up, accuracy and thoroughness are more important than rapidity in the other processes. Improvement in the power to observe is measured in the same manner by means of a graduated series of objects, such as a cyme, a composite head and a grass spikelet. This makes it possible to establish a norm for rate of improvement, and to measure the performance of each student against this as well as his original score. The relation to the latter is regarded as furnishing the best evidence of the student's progress, while the relation to the improvement norm of the group is taken as indicating the standard to be attained. Experimenting involves both observing and reasoning, and apart from these is measured in terms of the proper steps in organizing and executing an experiment. Remembering is best measured by written summaries of the knowledge to be put to use or tested by a project, though in terms of permanence it is also measured by the unannounced test in any field desired. Reasoning is more difficult to express in a norm, but this is successfully done by taking into account the steps necessary to marshal and contrast the evidence and to express it in a proper conclusion. Relating involves observing, remembering and reasoning, and has been most successfully measured in connection with the efficiency of flowers in pollination or with their relationship. The actual test is conducted by giving each student several flowers representing as many orders, which are to be compared as to structure and then arranged in a grouping that will most nearly express relationship based upon degree of similarity. This can be extended to families and genera, and with little modification to other organs, as well as to processes. Applying is the most difficult sub-process to measure in quantitative terms, as the transfer of conclusion, principle or method is rather a qualitative matter which often does not permit of degrees of success. Most difficult of all is the measurement of success in applying results to other courses and fields, and to every-day life, but no adequate investigation of this is possible until departments are willing to relinquish the teaching of their respective

subjects as such, and to co-operate intimately in the mental development of their students.

It is probable that the teachers who are willing to adopt new ideas and methods may be divided into three groups. The first includes those who in spite of their openmindedness need more evidence of the ineffectiveness of present courses and methods. This can best be obtained by a careful scrutiny of objectives and a comprehensive measurement of results. The second group comprises those who feel that changes are desirable but hesitate before such a complete one. For these there is no better plan than to continue the usual method in one section and to employ the inquiry method in another section of the same course. This has the disadvantage of confining the evidence to methods and of neglecting content, but it has proved conclusive as to the former in every case. The third group contains those who are convinced that a change in the direction of the student's interests is imperative and are endeavouring to find the way to make it effective. For these the problem is chiefly one of opportunity. In the case of the teacher working alone and responsible only to himself, at least so far as methods and content are concerned, there is no serious difficulty apart from that of material equipment. Moreover, this will prove transitory in nearly every case, as indicated by the fact that the acquisition of plant-house and garden has always followed the adoption of the process-inquiry method as a natural outcome of its fitness to human needs. In the case of departments, while the material equipment is often present, the staffs are none too often progressive and but rarely consistently so. In the latter case the method will approve itself as the only one possible for teachers who are at the same time investigators. Its chances of success are excellent also when all those directly concerned with elementary courses are in agreement about it, but when progressive members are in the minority or are handicapped by a conservative in authority, there is small reason to expect success. What can be hoped for at present is that progressive teachers here and there will adopt the process-inquiry method as just as basic and inevitable in teaching as in all other research, and that they will serve as centres for the spread of the spirit of inquiry to all teachers and students.

A handbook of the process-inquiry method is in preparation, but it will be several years before it can appear, and meanwhile the writer will regard it as a privilege to get in touch with those who are carrying on research in teaching or are contemplating it.

PERMEABILITY

By WALTER STILES

CHAPTER XIII

REVERSIBLE AND IRREVERSIBLE CHANGES
IN CELL PERMEABILITY

IT is commonly supposed that the permeability of the plant cell
is capable of undergoing changes, and statements to that effect,
and involving that point of view, are common in the literature of
the subject. But before discussing these changes it is necessary to
enquire what exactly is meant by the term "change in permeability."
In making this enquiry we are brought up at once against the difficulty
referred to in the first chapter, namely, our present very inadequate
analysis of the system involved. In the preceding chapters it has
already been necessary to call attention to the fact that the rate of
intake of a substance is not necessarily a measure of the permeability
of any or all of the cell membranes, as the rate of intake will depend
on the difference of concentration within and without the cell, and
so also on the position of the equilibrium attained in the intake. Nor
on the other hand can exosmosis be used as a measure of permeability.
To make the position clear let it be supposed that when a plant cell
is placed in a certain solution no diffusion out from the cell takes
place at all. Suppose the cell is now transferred to a second solution
into which a considerable exosmosis of substances takes place forth-
with from the cell. It would be generally said that the solute in the
second solution had brought about an increase in the permeability
of the cell, or of the protoplasm, or of the plasma-membrane. This
view is really based on the assumption that the protoplast or vacuole
is surrounded by a membrane which is changed physically or chemi-
cally by the solute in the external solution, so that the substances
within are now able to diffuse through the membrane. This might
indeed be the true explanation, but clearly others are possible. Thus
the solute in question might affect non-diffusible substances of com-
plex molecular constitution so that they broke down into a number

of substances of low molecular weight which can diffuse freely out of the cell. It is obvious that in such a case the change observed is not necessarily connected in any way with changes in permeability.

Nevertheless, it seems clear that substances may pass with different degrees of difficulty into or out from the cell under different conditions, even when the differences of concentration within and without the cell are the same, and it may be convenient to speak of any such difference which can be brought about by change in conditions as due to a change in permeability, although it must be emphasized that the term permeability is then not used in a strict sense (cf. Chapter I). Even then it is very questionable whether it is legitimate to speak of the permeability of the cell or of any part of it without reference to the substances concerned, for it is by no means certain that a change in permeability to one substance involves the same relative change in permeability to another substance (cf. Chapter V). In the following discussion the term "change of permeability" is used to indicate a change in the ease with which a substance will pass into or out from the cell unconnected with a change in the difference in osmotic pressure or of concentration within and without the cell. It should, however, be clearly recognised that in so doing the term permeability is used loosely and with no intention of accepting the point of view that the changes observed are entirely due to alterations in the permeability of a cell membrane.

Such changes may be reversible or irreversible. The latter appear always to be in the direction of increased permeability, with exosmosis of dissolved substances and, in the case of the turgid cell, of water, ending in complete loss of turgidity and in death. Such irreversible changes are produced by high temperatures, freezing and subsequent thawing, and toxic substances. It is possible they may be also brought about by rapid withdrawal of water resulting from immersion of a cell in a strongly hypertonic solution. The course of water loss during such an irreversible change in permeability is illustrated by the curves in Fig. II in Chapter X, showing the change in water-content of potato tuber immersed in solutions of ethyl alcohol of various concentrations. The exosmosis of electrolytes from the same tissue immersed in solutions of the same alcohol of various concentrations at a temperature of 20° C. is indicated by the curves in Fig. 16 which exhibit the increase in electrical conductivity (corrected for the presence of non-electrolytes) of 50 c.c. of external solution containing 20 disks of potato tissue, 17 mm. in diameter and 1·75 mm. thick. A comparison of the two sets of curves shows that the same concen-

trations of alcohol which bring about rapid exosmosis of water also bring about rapid exosmosis of electrolytes. It could be argued that the loss of water results from the loss of electrolytes and the consequent lowering of the osmotic pressure of the cell; in the present state of our knowledge it is safest to regard both as part of the phenomena of disintegration of the organisation of the cell characterising death.
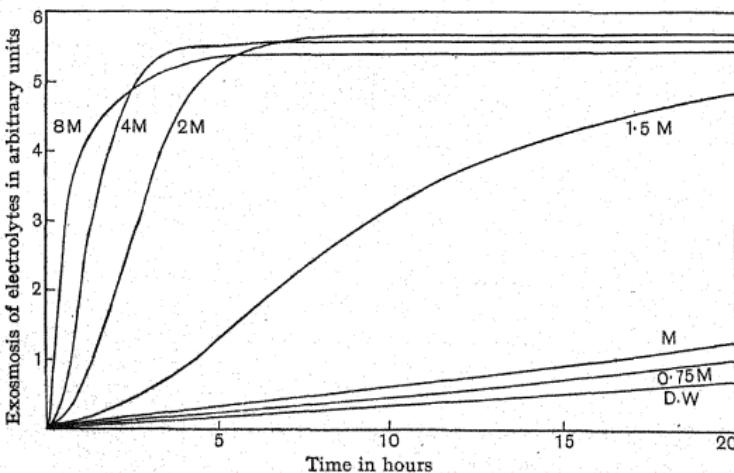
Fig. 16. Exosmosis of electrolytes from potato immersed in solutions of ethyl alcohol of various concentrations ($8M$ to $0.75M$) and in distilled water (D. W.). (After Stiles and Jørgensen.)

It was shown by Stiles and Jørgensen (1917 a) that the relation between the quantity of exosmosis and the time of action of a toxic substance can be expressed approximately by the equation

$$- B \log (1 - ks) = D \left(\frac{1}{A} e^{-At} + t - \frac{1}{A} \right),$$

where s is the concentration of the electrolytes that have exuded into the external solution after any time t and A, B, D and k are constants, this equation being that obtained when it is assumed that the rate of exosmosis at any time is proportional to the quantity of a substance in the cell which is decomposed by the toxic substance. The value of the constant A depends partly on the concentration of the toxic substance in the external solution; from the exosmosis-time curves obtained with different substances it is clear that whatever the relation between A and concentration may be it is not a linear one.

It has previously been mentioned that the electrical conductivity of tissue increases when acted upon by a toxic substance, the rate of increase giving a rough measure of the toxicity (Osterhout, 1912 *a*; Raber, 1920 *a*). Osterhout's elaborate theory of antagonism described in the last chapter is easily extended so as to explain the changes in electrical conductivity of tissue acted upon by a toxic substance (Osterhout, 1917 *e*).

The relation between concentration and toxicity was investigated by Wolfgang Ostwald (1907) in the case of a freshwater animal, *Gammarus*, this worker coming to the conclusion that the life-time *t* of the animal in a solution of concentration *C* is given by the equation

$$\frac{1}{t} = kC^{p},$$

where *k* and *p* are constants.

The similarity of this equation to the adsorption equation led Ostwald to regard $\frac{1}{t}$ as proportional to the quantity of toxic substance adsorbed. The law has been shown to hold by Weevers (1912) for the toxic action of chloroform vapour on cells of the root of the red beet, the exosmosis of the red pigment being taken as an indication of the death of the cells. The same worker found (1914) that this relation only held within narrow limits in the case of the poisoning of the same cells with solutions of quinine hydrochloride and chloral hydrate. With more dilute solutions the cells live longer than the time indicated by Wolfgang Ostwald's equation. A similar divergence was observed by Szücs (1912) in his experiments on the time required for seedlings of *Cucurbita Pepo* to absorb enough copper sulphate to kill them, and by Nothmann-Zuckerkandl (1912) in experiments on the action of alcohol on protoplasmic streaming in *Vallisneria*.

Several arguments against Ostwald's conclusion have been put forward by Szücs, who showed that the former writer's view that the time taken to kill is inversely proportional to the quantity of poison adsorbed involves several assumptions. It assumes in the first place that equilibrium between the quantity adsorbed and the outer medium appears instantaneously, for otherwise the life-time will be increased. In the second place it is assumed that small quantities have the same effect ultimately as larger ones, but require more time for the effect to be observed. Thirdly, it is assumed, since the adsorption equation only refers to an equilibrium condition, that the toxic effect is only produced when the equilibrium is reached.

These various assumptions are unjustified by the facts. With regard to the first assumption, equilibrium in the intake of copper sulphate was shown by Szücs to come about very slowly. With regard to the second assumption, it is not true that small quantities have the same effect as larger ones if they are given the necessary time to act, for the intake of small quantities of a poison may not lead to death. That this is not due to the washing out of the poison into the surroundings is shown by the fact that it takes place with heavy metals which are supposed to form irreversible compounds with the cell proteins, and that if plants are grown with their roots in a poisonous solution and removed from it before the lapse of a definite critical time they survive. Finally, with regard to the third assumption, Szücs found that with very poisonous substances death may occur before the equilibrium condition is reached.

For these reasons Szücs held that Ostwald's theory could not be maintained in its original form. Szücs himself appeared to incline to the opinion of Morawicz (1910) that the same quantity of material must be absorbed to bring about death. Szücs himself found the time required for seedlings of *Cucurbita Pepo* to absorb enough copper sulphate to kill them, by maintaining their roots in the copper sulphate solutions for definite times and then testing their vitality by examining their power to react to the stimuli of light and gravity. He then found that where t is the time required for the seedlings to take up enough copper sulphate to inhibit the geotropic reaction,

$$\frac{1}{t} = kC^p,$$

k, C and p having the signification previously assigned to them.

But these considerations on the relation between the concentration of toxic substances and time taken to kill, do not help us very much in determining the influence of concentration in bringing about a change in permeability. They have, however, this bearing on our subject, that, according to the view involved in Wolfgang Ostwald's theory, a substance that produces irreversible changes in permeability leading to death, would do so at once, whereas according to the view of Szücs the action of the toxic substance might only become irreversible after a definite critical period of immersion, varying in length with the concentration according to the equation cited above.

It is at least possible that all changes resulting in increased permeability of the cell are reversible if the condition producing the change is not allowed to act for more than a certain time.

It is generally supposed that all living cells in their normal condition undergo changes in regard to the ease with which substances pass into and out from them. These are sometimes spoken of as functional (Bayliss, 1915) changes in permeability. As these changes are reversible they can obviously take place in either direction, that is, so as to increase or diminish the capacity of substances to pass into or out from the cell. In the former case, if the action is prolonged, we may expect the change to become ultimately irreversible.

Factors which may bring about changes in permeability are those of the environment of the cell, namely, temperature, light and the composition of the external medium. The changes produced by various factors will now be considered.

EFFECT OF TEMPERATURE ON PERMEABILITY

The influence of temperature on the rate of intake and exosmosis of water by plant cells, and on the permeability of cells to water, has already been considered in Chapter X, and the influence of temperature on the rate of intake of dissolved substances has been discussed in Chapter XII. The effect of temperature over the range 0° C. to 30° C. is to increase the rate of intake of both water and dissolved substances, although the magnitude of the temperature effect varies considerably. A good case can be made out for regarding the change in the rate of intake of water into the vacuolated cell with change in temperature as due to a change in permeability of the protoplasm. That the same is the case with dissolved substances is not in any way evident. Within the range of temperatures indicated, changes related to changes of temperature are certainly reversible, even if the conditions are continued over comparatively long periods of time, but if the temperature rises too high, or if the tissues are allowed to freeze, the tissues or cells quickly disorganise owing to other changes which as far as we know may have nothing to do with permeability.

EFFECT OF LIGHT ON PERMEABILITY

The influence of light on the intake and excretion of salts has already been dealt with in the preceding chapter. Such changes in relation to light appear undoubtedly to be reversible. Here again they can only be spoken of as changes in permeability if the latter term is used in a loose sense.

Haupt (1902) concluded that light influences the permeability of the cells of extra-floral nectaries in *Euphorbia* and *Vicia*. He found excretion of sugar only took place from the cells when illuminated,

while in the dark sugar was absorbed. Since the behaviour is observed
in plants that have been maintained for days in absence of carbon
dioxide it is concluded that the behaviour is not related to carbon
assimilation, but is due to changes in the permeability of the cells
to sugar. But this explanation appears to me hardly to be in agree-
ment with the facts, for a mere change in permeability of a mem-
brane will not account for the reversal in direction of the movement
of the solute. A change in the physical or chemical constitution of
the cell contents, brought about by a photochemical reaction, appears
a much more credible explanation of the observed facts.

INFLUENCE OF DISSOLVED SUBSTANCES ON PERMEABILITY

In addition to the antagonistic effects described in the last chapter
there is a certain amount of evidence that substances in solution
external to plant cells influence the rate at which substances pass into
or out from plant cells. The action of toxic substances in this respect
has already been discussed. Apart from these undoubted cases there
are a number of observations on record which are alleged to indicate
the influence of dissolved substances in altering permeability. Thus,
Wächter (1905) found that the passage of sugars out from the bulb
scales of the onion was prevented by the presence of potassium nitrate
in the liquid external to the plant. A rather similar observation is
that recorded by Bayliss (1915) to the effect that while a 0·31 M
solution of sodium chloride brings about exosmosis of the pigment
from the cells of the root of the red beet, no such exosmosis takes
place into a solution containing the same concentration of sodium
chloride and also 0·17 per cent. of calcium chloride. Both these
observations can be explained on the basis of a reduction of permea-
bility of the cell to a cell constituent, the reduction being brought
about by the presence of a solute in the external solution. In the
second case the observation can be brought into line with the
antagonistic effects noted in the last chapter.

Fluri (1909) thought he had obtained evidence that aluminium
salts brought about an increase of permeability of plant cells to
glucose and a number of salts. When filaments of *Spirogyra* were
immersed for two or three days in a 0·01 per cent. solution of alumi-
nium sulphate or nitrate, the cells were no longer plasmolysable in
hypertonic solutions of glucose or a number of salts. Fluri explained
this as due to the increased permeability of the cell to these sub-
stances. On transferring the filaments back to water the cells recover
their original condition, so that the effect is reversible.

However, investigations of Stoklasa (1911) and others, and particularly the critical research of Szücs (1913) on this subject, indicate that this explanation is not correct, for if it were, the following consequences should result.

1. Cells plasmolysed in a hypertonic solution should become deplasmolysed after addition of an aluminium salt to the external solution.

2. The increased permeability of the protoplasm should condition increased exosmosis and consequent loss of turgor.

3. As they can enter the cell more rapidly, toxic substances ought to exhibit their effects more rapidly in presence of aluminium salts.

4. Substances which are microscopically recognisable ought to be clearly discernible by microchemical tests if they enter the cell in such amount that plasmolysis is prevented.

5. The rapid entrance of strong solutions ought to have toxic effects which would condition exosmosis.

Szücs could observe none of these consequences of Fluri's theory of the action of aluminium ions. On the contrary, he found by centrifuging filaments of *Spirogyra* treated with an aluminium salt, that the action of aluminium ions is to fix the protoplasts, which remain in position under this treatment, while normal living protoplasts collect in the ends of their cells. Aluminium chloride, sulphate, nitrate and acetate all appear to behave in the same way, while yttrium and lanthanum salts also appear to have the same action. On returning the cells after treatment to water, they recover their original state. There is no doubt that the effect is reversible. If the aluminium ion is allowed to act for a longer time, and if it is in sufficient concentration, the protoplast becomes "loosened" again, and then collects in the ends of the cell on centrifuging, in the same way as in a normal living cell. The fixing action can be observed in *Spirogyra* immersed for only a minute in a solution of aluminium nitrate as dilute as $0.0064\ N$. The greater the concentration the more rapidly both the fixing action and the subsequent "loosening" take place. It should be noted that aluminium ions do not exert this fixing action on anthocyanin-containing cells of a number of different species examined by Szücs.

Harvey (1911, 1913), from experiments in which cells were first impregnated with neutral red (cf. Chapter XI), concluded that sodium hydroxide does not readily enter cells of *Elodea*, *Spirogyra*, *Paramœcium* and eggs of various echinoderms, but that sodium salts in the external solution render the cell permeable to sodium hydroxide.

It is not clear, however, how far this result is to be attributed to toxic action.

S. C. Brooks (1916 *b*) treated strips of peduncle of *Taraxacum* with solutions of respectively 0·22 *M* sodium chloride, 0·17 *M* calcium chloride and 0·05 *M* cerium chloride for 20 or 25 minutes, then transferred the tissue to distilled water and measured the increase in electrical conductivity of the latter. He assumed that exosmosis during the first 30 minutes is due to diffusion into the water of the salt previously absorbed, and that subsequent exosmosis can be regarded as due to exosmosis of electrolytes from the protoplasm. Since it was found that after 30 minutes exosmosis from tissue that had been treated with calcium chloride was less than that from tissue which had not been in contact with any salt solution, it was concluded that calcium chloride brings about a decrease in permeability of the tissue, while as from tissue treated with sodium chloride exosmosis after 30 minutes was greater than from the control which had not been in contact with salt solution, it was concluded that sodium chloride brings about an increase in permeability. Cerium chloride was regarded as bringing about first a decrease in permeability which is followed by an increase.

To the present writer these conclusions appear of little value for the following reasons. The recorded observations are few in number, the recorded differences are small, and it is not shown that they lie outside the limits of experimental error. It is necessary to show this, especially as electrical conductivity can be used only as an approximate, but not as an accurate, measure of the exosmosis of electrolytes, while it is unlikely that diffusion of the experimental salt out of the tissue should end, and that of other substances in the cells begin, at any particular moment, or at the same time with different salts.

Experiments with the tissue tension method and the diffusion method described in the last chapter led Brooks to similar conclusions, namely, that salts of univalent kations (and also sucrose) increase permeability, while salts of divalent and trivalent kations at first bring about a decrease in permeability. But these methods also are not free from serious objections, and the legitimacy of the conclusions drawn appears to the present writer very doubtful (cf. Chapter XI).

As indicated in the last chapter Osterhout (1912 *a, b*) found that thallus of *Laminaria* when transferred from sea water to sodium chloride having the same conductivity as sea water, underwent a gradual loss of electrical resistance until the tissue was quite dead.

A number of salts were found to act in the same way, namely, the chlorides of potassium, cæsium, rubidium, lithium, ammonium and magnesium[1]; sodium bromide, iodide, nitrate, sulphate and acetate. On the other hand the salts of bivalent metals examined (magnesium, calcium, barium, strontium, manganese, cobalt, iron, nickel, zinc, cadmium and tin) produce first a rise in resistance, then a fall (Osterhout, 1915 *d*); the same is the case with salts of trivalent and tetravalent metals (Osterhout, 1915 *e*). The same effect is produced by ether, chloroform, chloral hydrate and alcohol (Osterhout, 1913 *a*, 1916 *f*), by potassium cyanide (Osterhout, 1917 *a*) and by sodium taurocholate (Osterhout, 1919 *b*).

On the assumption that electrical conductivity is a measure of permeability these results are interpreted as indicating that the substances of the first group bring about an increase in the permeability of the cell, while those of the latter bring about first a decrease in permeability followed by an increase. For the same reasons small quantities of sodium hydroxide are held to increase permeability (Osterhout, 1914 *g*) while hydrochloric acid is considered to produce rapid decrease of permeability followed at once by rapid increase (Osterhout, 1914 *h*).

If the tissue is only allowed to remain in the single salt solutions for a short time and is then returned to sea water, the original resistance of the tissue is regained. This is regarded by Osterhout (1912 *b*, 1915 *b*) as indicating reversible changes in permeability.

Reasons why electrical conductivity cannot be regarded as an accurate measure of permeability have already been advanced in Chapter XI and need not be repeated here. Also it seems to the present writer meaningless to speak of the permeability of the cell as a quantity with a definite value which can be accurately measured, unless the substance, the penetration of which is in question, is also stated, for there is no evidence that if the permeability of the cell to one substance changes, its permeability to all other substances changes in the same proportion. Osterhout's theory of antagonism described in the last chapter has been extended to include reversible changes of electrical conductivity (Osterhout, 1920 *a*, *b*), but as it is highly speculative, it will not be dealt with further in this place.

Tröndle (1920) investigated the effect of acids on the permeability to sodium chloride of the cells of the leaf of *Buxus sempervirens* by a plasmolytic method in which were recorded the concentrations of

[1] But magnesium salts appear in Osterhout's second group.

sodium chloride in which deplasmolysis just took place after various times of immersion. It was found that after immersion for five minutes in 0·01 N oxalic acid or 0·005 N hydrochloric acid the plasmolysing concentration of sodium chloride rose more rapidly than after a preliminary immersion for five minutes in tap water. The rise in the value of the limiting plasmolysing concentration is attributed to the absorption of sodium chloride and hence it is concluded that dilute acid has the effect of increasing the permeability of the cells in question to sodium chloride.

The observation of Puriewitsch (1898) that exosmosis of sugars or other soluble food reserve substances from storage tissue is inhibited in absence of oxygen or in presence of anæsthetics, is regarded by Livingston (1903) as probably a case of the influence of a dissolved substance on the permeability of a cell membrane. Yet to me Puriewitsch's original explanation, namely, that the absence of oxygen or the presence of anæsthetics influences the enzymes present so that the stored food material is not converted by enzyme action into a diffusible form, appears at least as probable. Livingston finds it difficult to understand how enzyme action could enter into the question in cases where the food reserve is in a soluble form as in the case of sugar in onion and beet root. It might, however, be the case that there is here a transformation of disaccharides or more complex sugars into the more easily diffusible hexoses or pentoses.

The influence of various narcotics on the absorption of salts by leaf cells was examined by Tröndle (1920) by the plasmolytic method used by him to test the action of acids on permeability. Assuming the legitimacy of the method, which is in doubt, it was found that previous immersion for 20 minutes in 0·5 per cent. chloral hydrate reduced the rate of absorption of sodium chloride by leaf cells of *Buxus sempervirens*, while immersion of the same cells for the same time in 1 per cent. chloral hydrate or 3 per cent. (by volume) ether at about 15° C. completely inhibited the intake of salt. The absorption of potassium chloride by leaf cells of *Acer platanoides* was inhibited by previous immersion for 15 minutes at about 21° C. in 1 per cent. chloral hydrate, and similarly the absorption of sodium nitrate by leaf cells of *Lupinus albus* was inhibited by immersion for 20 minutes in the same solution. From these results Tröndle concludes that narcotics reduce the permeability of cells to salts, even to complete impermeability.

EFFECT OF CHANGES IN TURGOR

To account for the exudation of water or solution from living cells in the case of water pores and nectaries, and in the phenomena of bleeding and root pressure, it has been suggested that a change in the permeability may be involved which is brought about when the turgor pressure has reached a certain amount. It is certainly conceivable that a stretching of the protoplasmic envelope might render it more permeable. Livingston (1903) thus thinks that as water passes into the cells in question the turgor pressure may rise to a critical point when a change in permeability takes place as a result of which cell sap exudes through the protoplasm. As a result of this the turgor pressure is reduced and the cellulose wall contracts. If the change in permeability is uniform throughout the whole of the protoplasm, exudation will only take place to any extent into the cavity of the pore of the water stoma or the cup of the nectary, as the turgor pressure of the surrounding cells will oppose the passage of water into them.

After the cell wall and protoplasm have contracted so that turgor pressure is below the critical value, the protoplasm regains its semi-permeable property, water passes in from the neighbouring cells, and the turgidity increases until the critical value of the turgor pressure is again reached. During this period reabsorption of the water previously extruded is prevented because evaporation has concentrated the extruded solution so that it has now a higher osmotic concentration, and would therefore tend to extract water from the cell. It must be supposed on this (and perhaps not only on this) view of the action of water pores and nectaries, that the solutes lost from the vacuole in exudation are replaced by secretion in the protoplasm.

A similar mechanism can be supposed to act in the case of the exudation of water from cut stems and leaves, and in the phenomenon of root pressure.

It must be emphasized that this theory was only put forward by Livingston as a tentative one. It is possible that no change in permeability is involved in the phenomena of exudation, bleeding and root pressure, and references to theories in which this latter point of view is taken have already been noticed in Chapter IX.

EFFECT OF WOUNDING

Tröndle (1921) investigated the effect of wounding on the permeability of cells of the young roots of *Pisum*, *Vicia*, *Lupinus* and *Allium* by the method used by him to investigate the effect of acids and narcotics on permeability, and already mentioned in this chapter,

that is, by determining the limiting concentration of sodium nitrate and potassium chloride required to produce plasmolysis of these cells after the lapse of different periods of time. Assuming the legitimacy of the method, which is doubtful, it was found that injury decreased the rate of intake of the salts employed for plasmolysis, whence it is concluded that wounding decreases the permeability of the protoplasm of these cells to the salts in question.

SEASONAL CHANGES IN PERMEABILITY

Fitting (1915) found by the deplasmolytic method used by him, that salts were absorbed by the cells of the leaves of *Rhœo discolor* much more slowly in winter than in summer; he found the same thing with glycerol (1919).

It is evident from what has already been written that the cell may vary under different conditions with regard to its capacity for absorbing or excreting both water and dissolved substances. In the case of the vacuolated cell such changes in the rate of the passage of water from external liquid to vacuole or *vice versa* may, with constant difference in osmotic concentration of the internal and external liquids, be regarded as due to changes in the permeability of the cell membranes (cell wall + protoplast) to water, although even this rests on the assumption of the complete correctness of the simple osmotic view of the plant cell, which has already been shown to be inadequate (cf. Chapter IX).

The question of changes in permeability to dissolved substances is a much more difficult one. That changes in permeability are spoken of at all in this connexion is due to the acceptance of the membrane theory, and it must not be forgotten that other explanations are possible which do not involve passage of substances through a membrane (Moore and Roaf, 1908). With regard to the irreversible changes in permeability leading to the death of the cell, Szücs (1912) is of opinion that the so-called "increase in permeability" brought about by narcotics and other compounds, and indicated by exosmosis of tannin from plant cells and the pigment from red blood corpuscles, has nothing to do with permeability because the phenomena observed are not connected with vital processes but are due to post-mortal changes.

Yet even if the exosmosis resulting from the action of toxic substances is not in the first place attributable to post-mortal changes,

it does not follow that such exosmosis is due to increased permeability of the cell membranes to the solutes of the cell. It may, for instance, result from a breaking down of complex and indiffusible substances into simpler and diffusible ones. Similarly, the so-called reversible changes in permeability may be attributed to reversible changes in molecular associations rather than to changes in the permeability of the cell membranes. Which is the correct view cannot be decided in the present state of our analysis; it can only be determined by further work.

CHAPTER XIV

THEORIES OF CELL PERMEABILITY

IT will be clear from the review in the preceding chapters of the data at present available with regard to the permeability of plant cells, that our information is far from complete, and, as far as quantitative data are concerned, fragmentary. Nevertheless, in spite of the insufficiency of information, there is no shortage of theories of cell permeability. It is one of the weaknesses of the theories of cell permeability that they are for the most part not even based on the whole of the scanty information available, but only on one particular set of observations which fit the particular theory. Under these circumstances perhaps none of the theories are of any great value, and no attempt will be made here to summarise in any detail all the theories that have been put forward to account for certain facts of cell intake or excretion. Those theories will chiefly be considered which have come into prominence during recent years, and which, in the opinion of the writer, may be perhaps of some use, in that they may stimulate to further work.

Two questions which have been discussed in earlier chapters are obviously of first importance in regard to the mechanism of permeability in living cells. These are (1) the purely physico-chemical question of the permeability of membranes discussed in Chapter V, and (2) the plasma-membrane discussed in Chapter VIII. From the discussion on the plasma-membrane it must be admitted that the evidence for the presence of a membrane which acts as a semipermeable membrane separating the bulk of the protoplasm from the external solution (supposed to be present as such in the cell wall) is not by any means convincing, although to regard the whole body of the protoplasm in the vacuolated cell as a more or less semi-

permeable membrane separating the external solution has more in favour of it.

Most of the propounders of theories of cell permeability assume a semi-permeable plasma-membrane forming the external layer of the protoplasm in all cells, and if this were the case, it might perhaps be possible to find an explanation of cell permeability that would hold in all cases. But if this supposition is erroneous it is clear that we cannot expect a theory which explains the passage of substances from the outside of the cell through the protoplasm into the vacuole, necessarily to explain also the intake of the substance by the protoplasm, that is, the membrane.

To those who believe in a more or less semi-permeable plasma-membrane limiting the protoplasm externally, the three theories of membrane permeability described in Chapter v are obviously applicable to the penetration of substances into the cell, and we do actually find that all three theories, the sieve theory, the solution theory and the chemical combination theory, have been put forward in some form or another as theories of cell permeability. But to those who do not accept the presence of such a plasma-membrane, these theories in their simple form obviously cannot account for the intake of substances by meristematic cells or excretion from them, while in the case of vacuolated cells they can at most only account for the passage of substances into the vacuole and not for their uptake by the protoplasm. This essential dependence of theories of cell permeability on belief or disbelief in the existence of a plasma-membrane of restricted permeability has not always been sufficiently emphasized in discussions on the mechanism of absorption by cells, even if it has been fully realised.

Since some theories of cell permeability rely for some considerable measure of support on observations on the intake of dyes by living cells ("vital staining") it will be as well to preface a consideration of the individual theories with a brief summary of the main facts of dye intake.

The work of Pfeffer and subsequent investigators suggested that while plant cells rapidly absorb basic dyes, acid dyes are in general absorbed not at all or only to a slight extent. Overton (1899, 1900), whose theory of permeability will be discussed later, came to the conclusion that only dyes soluble in lipoid substances enter the cell. Basic dyes are, however, on the whole, soluble in lipoid substances, while acid dyes are not. It was later found by Höber and Kempner (1908) and Höber and Chassin (1908) that many acid dyes are taken

up by the epithelial cells of the kidney and accumulate there; nevertheless, some acid dyes are not taken up. Höber and his co-workers came to the conclusion from their experimental results that when a dye is not absorbed by the epithelial cells of the kidney it is highly colloidal; whereas if a dye is only slightly colloidal or semi-colloidal it is easily absorbed.

In the next year Ruhland (1908 *a*, *b*) came to the conclusion from experiments with *Spirogyra*, that whatever the degree of dispersity of the dye, the cells in question readily absorb a basic dye but not an acid dye. In the same year Höber (1909) examined the capacity of 34 dyes to stain the epithelial cells of the kidney and came to the same conclusion as he had done previously, namely, that those dyes which do not stain these kidney cells are unable to do so on account of their low degree of dispersity. He rightly pointed out that his results only hold for kidney cells and were not necessarily applicable to cells in general.

In further work which will be dealt with in some little detail later in this chapter, Ruhland (1912 *b*, 1913 *a*, *b*, *c*) examined the intake of a great number of dyes, both acid and basic, by a different method and as a result changed his point of view and now holds that it is solely the size of the particles of the dye which determines whether the dye is taken up or not.

The importance of the hydrogen-ion concentration of the cell contents is emphasized by Bethe (1916, 1922) and Rohde (1917), who find that the capacity of living cells to absorb dyes depends in high degree on the reaction of the external medium and of the cell contents. Acid reaction in the interior of the cell favours the accumulation of acid dyes and acts against staining by basic dyes, while inversely, basic dyes are extraordinarily strongly absorbed by cells with an alkaline reaction while acid dyes scarcely stain such cells at all. Bethe (1922) adduces in support of this view experiments in which basic and acid dyes and one amphoteric dye were allowed to diffuse through parchment paper under different conditions of hydrogen-ion concentration. With acid dyes diffusion is furthered by an acid reaction and reduced by an alkaline one; with basic dyes the reverse is the case. Similarly, if the dye is allowed to diffuse from an aqueous solution through parchment paper into a protein, the dye accumulates in the solution when an acid dye diffuses into an acid protein sol or when a basic dye diffuses into an alkaline protein sol, but not when the reaction of the sol is acid and the dye a basic one, nor when the reaction of the sol is alkaline and an acid dye is employed.

With these prefatory remarks we are now in a better position to consider some of the theories of cell permeability.

THE ULTRAFILTRATION THEORY

The ultrafiltration theory of cell permeability appears to be a direct application to the cell of the sieve, theory of membrane permeability. In its present form it is founded on the work of Küster (1911) and Ruhland (1912, 1913 a, b, c, 1914) on the staining of various plant cells by a considerable number of dyes. The method chiefly employed was introduced by Küster and consists in placing the cut surface of shoots in a solution of dye so that the solution is carried through the vascular bundles from which the dye, if it is capable of penetrating living cells, will pass into the latter. Using this method Ruhland examined the penetration of 89 acid dyes, chiefly into the tissues of young plants of *Vicia Faba*, and came to the conclusion that a complete parallelism exists between penetrability and diffusivity of the dye, the latter being examined by following the diffusion of the dye through a gelatin gel. The diffusivity is supposed to run parallel to the degree of dispersion (cf. Chapter IV), whence it is concluded that with acid dyes the capacity of penetrating into plant cells depends entirely on the degree of dispersion of the dye.

Ruhland also examined the intake of 30 basic dyes by epidermal cells of the bulb scales of *Allium Cepa* and by cells of *Spirogyra*, and found that of these a few, namely, Victoria blue *R* and *B*, Basler blue *R* and *BB*, gallamin blue and night blue, were not absorbed, while two others, diazine green and Victoria blue *R*, were only absorbed slowly. All these dyes were also found to diffuse slowly through a gelatin gel.

In some cases the intake of acid dyes by cells of the bulb scales of *Allium Cepa* and of the pith of the stalk of *Helianthus annuus* could be made plain after immersion of the tissue in the dye by plasmolysing with a strongly hypertonic solution of sugar or a salt so that a big contraction of the protoplast resulted; with a consequent intensification of the colour. Küster (1918) has also obtained similar results in regard to the intake of acid dyes (acid fuchsin, light green *FS* and orange *G*) by cells of the pith of *Coleus hybridus*.

The fact that basic dyes accumulate much faster than acid dyes is regarded by Ruhland as not related to permeability, but to be due to the combination of basic dyes with tannic acid (cf. Chapter XI) so that a considerable apparent difference in concentration of the dye on the two sides of the plasma-membrane is maintained.

Ruhland thus comes to the conclusion that the entrance or non-entrance of a substance into the cell is related entirely to the magnitude of its molecules or molecular aggregates, the plasma-membrane acting exactly as an ultra-filter.

Ruhland's theory has been subjected to criticism by Höber and Nast (1913). It will be recalled that Ruhland found a few highly colloidal basic dyes that would not penetrate the cells examined. Höber and Nast, on the other hand, found that all these dyes will stain living animal cells with the exception of gallamin blue, and this dye, according to Höber and Nast, is an acid and not a basic dye. These dyes are not very soluble and are easily precipitated by electrolytes, and Höber and Nast consider it possible that the dyes were precipitated before they reached the plasma-membrane, possibly by the electrolytes in the cell walls. In any case the ultra-filter theory is not supported by the work of Höber and his collaborators on animal cells.

The ultrafiltration theory has been criticised by Collander (1921) from another point of view. The last-named writer points out that the cells which take up the dye when Küster's method is used are those in the neighbourhood of the vascular bundles, and Collander's work on the intake of sulphonic acid dyes has shown that these cells may have a quite abnormally high capacity for absorbing acid dyes. If the theory were sound all cells should absorb acid dyes of a high degree of dispersion, but this has been shown by Collander not to be the case, while the earlier experiments of Ruhland (1908 *a*, *b*) himself indicate the same thing. It is clear that the ultrafiltration theory cannot be accepted as a complete and general theory of cell permeability.

THE LIPOID THEORY

The lipoid theory of cell permeability propounded by Overton (1895, 1896, 1899, 1900, 1901) is a solution theory of permeability, assuming as it does that the penetrating capacity of different substances runs parallel with their solubility in lipoid substances of which the plasma-membrane is supposed to be composed.

Overton founded his theory on experiments with a wide range of tissues and a large number of different substances including many dyes and other organic compounds of different groups.

As already mentioned, most acid dyes are insoluble in lipoid substances, but Ruhland states that some sulphonic acid dyes are soluble in lipoids and do not stain living cells, and three acid dyes, cloth red 3*GA*, true red *A* and wool violet *S*, were found by Höber

(1909) to be soluble in lipoid substances, but yet did not stain most cells. Höber showed that wool violet may be actually absorbed but undergoes a change in the cell resulting in decolorisation, as this can be effected by treatment of the dye with fresh frog's liver. Küster (1911) found all these dyes were absorbed by some, although not all plant cells. However, Collander has shown that cells of sugar beet and *Elodea* do not absorb wool violet S with any rapidity. There appears to be some doubt whether this dye should be regarded as a typical lipoid-soluble dye, as an experiment made by Collander of the partition of the dye between water and a saturated solution of cholesterol in benzene resulted in the dye going in greater quantity to the water.

Höber also points out that in another group of acid dyes, the phthaleins, there are a number which are soluble in lipoid substances but which do not stain living cells, namely, rose bengal, cyanosin, erythrosin and gallein.

Ruhland also states that new blue R is a dye insoluble in lipoid substances, but which stains living cells. Even methylene blue, one of the most easily absorbed dyes, prefers water to lipoids (Loewe, 1912). However, Höber and Nast found new blue R was soluble in a solution of cholesterol in turpentine. The absorption of many sulphonic acid dyes by certain plant cells as shown by Küster's method, appears to tell against the lipoid theory, as these dyes are in general insoluble in lipoid substances.

It is clear that a simple lipoid theory will not explain the behaviour of all cells to all dyes, but the principal difficulty arises from the intake of inorganic salts which are as a rule insoluble in lipoid substances. Consequently the theory has to admit that substances insoluble in lipoids may, under certain circumstances, be able to penetrate into the cells. Hence we find solution theories put forward in which the plasma-membrane is regarded as a mosaic (Nathansohn, 1904 *a*) or a colloidal complex (Lepeschkin, 1910 *a*, *b*, 1911 *a*, *b*, *c*) (cf. Chapter VIII) in which lipoid substances are present, by solution in which dyes, narcotics and other lipoid-soluble substances enter the cell, and in which there is also an aqueous phase through which water-soluble substances such as inorganic salts, acids and bases, and sugars, are able to diffuse into the cell.

The lipoid theory is at best an imperfect theory, only professing to indicate, as Collander puts it, what substances will enter all cells easily under all conditions. But even thus restricted the theory appears to break down, for, as already indicated, there are substances

soluble in lipoid substances which have been found not to enter living cells.

Boas (1921, 1922) made observations on the action of saponins on yeast cells and concluded that his results support the lipoid theory. He found that saponins in low concentrations bring about an increase in the rate of fermentation by yeast, although in high concentrations fermentation is retarded. This action is explained on the ground that saponins attack lipoids in the plasma-membrane so that the permeability is greatly increased and because of this sugars can be more rapidly fermented. The argument is not very convincing.

A modification of the lipoid theory recently proposed by Nirenstein (1920) as a result of work on *Paramœcium caudatum*, need not detain us long. According to this theory the living cell behaves as if it were a lipoid solvent containing a certain amount of fatty acid and organic bases soluble in fats, which between them are responsible for the uptake of substances, acid dyes, for example, being absorbed by the bases, such as diamylamine, in which they are soluble, and basic dyes by the fatty acids, such as oleic acid, in which they are soluble. Collander points out that this theory is not directly applicable to plant cells because accumulation of dyes takes place in the cell sap, but it might be possible to regard the plasma-membrane as possessing the solvent properties of *Paramœcium* protoplasm. However, Collander examined seven of the acid dyes which Nirenstein had found soluble in diamylamine and which stain living *Paramœcium,* but none of these dyes were taken up to any extent by plant cells. Nirenstein's theory is thus quite unacceptable in regard to plant cells.

THE COLLOID PRECIPITATION THEORY

A theory that permeability of protoplasm to any particular salt is dependent on the capacity of the salt to precipitate the colloids of the protoplasm has been put forward recently by Kahho (1921 *d*). This worker finds (1921 *a, d*) that the influence of salts on the coagulation of cell colloids by heat runs parallel with the penetrability of the salts as determined by the plasmolytic or tissue extension methods. The series obtained when kations and anions are arranged in order of their penetrability have been stated in the last chapter. These series are the reverse of the lyotropic series indicating order of capacity for precipitating proteins. If kations and anions are arranged in order of toxicity or of capacity for coagulating plant protoplasm, they fall into the same reverse lyotropic series (Kahho, 1921 *b*) while kations of the heavy metals appear to conform to the

same rule (Kahho, 1921 c). Kahho explains all these relations on the view that toxic action depends on power of penetration, while power of penetration depends in inverse fashion on capacity for coagulating certain of the cell colloids, probably the lipoid constituents of the protoplasm. Thus the reason why a calcium salt reduces the toxicity of a sodium salt is that the calcium coagulates the lipoid constituents of the limiting layer of the protoplasm, which according to Hansteen-Cranner (1919, 1922) penetrates into the interstices of the cellulose-pectin ("cellulose-hemicellulose") colloidal network of the cell wall. This coagulation of the lipoid constituents renders the outer layers of the protoplasm less permeable to the salts in the external solution so that the entrance of these salts with their consequent toxic action is prevented and the coagulation of the proteins of the protoplasm is thereby prevented.

This theory is attractive and Kahho marshals his own experimental data well in its support and adduces a number of observations by other workers as evidence on behalf of his theory. It must nevertheless be admitted by an unprejudiced critic that the experimental basis of the theory is still rather frail, the essential facts, namely, those relating to the actual penetration of different salts, having been obtained by the tissue extension method of Lundegårdh, the validity of which, for reasons advanced in an earlier chapter, is open to a certain degree of doubt, and which should certainly be confirmed by means of other methods of determining salt absorption. Considerably more work on the location and properties of the cell colloids in the actual species used would also appear desirable.

THE

NEW PHYTOLOGIST

ON THE PHENOMENA ATTENDING SEASONAL CHANGES IN THE ORGANISATION IN LEAF CELLS OF *PICEA CANADENSIS* (MILL.) B.S.P.

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CONTENTS

INTRODUCTION

OBSERVATIONS have been carried out by us for several seasons on the periodic changes which occur in the organisation of the cells and the physical conditions of the saps of winter evergreens in Northern Alberta. On account of the long winters and the extremely low temperatures to which the leaves are resistant, observations in N.W. Canada are of interest; for as far as we are aware no observations have been carried out on evergreen leaves under these conditions.

The work of Lidforss[1] on the winter evergreens of Sweden although containing much information as to the changes in the reserve materials and the causes of immunity to cold, does not deal with the physical conditions of the sap; in addition the length and severity of the Swedish winter does not compare with the winters of Northern Alberta.

In a previous paper[2] four native plants were examined throughout the winter, attention being directed to the osmotic pressures,

electrical conductivities and sugars of the sap. Records were also made of the structure of the cells in the leaves of winter evergreens during the winter period.

The present paper deals with the same features during the spring conversion from the winter condition, particularly with regard to the causes affecting conversion.

METEOROLOGY

A brief description of the conditions of the winter may be of interest. The daily temperatures from January 1 to April 30 are shown on page 227. The winter of 1919–20 was longer than usual. Snow and zero (F.) weather began in the third week of October. Long and frequent snowfall resulted in a covering of over 8 feet in March and the soil was not uncovered until the second week in April. In some districts the ground was still snow-covered at the middle of May. Winter weather conditions thus overlap the seasonal changes in the leaf cells of Picea during this year. This is a point of great interest, as the change from winter to summer conditions started during the cold spell early in April when minimum temperatures of − 14° F. were experienced and when the maximum was always below 30° F.

Measurements from the graph (p. 227) indicate that of the area enclosed by the minimum temperature curve only 0·4 per cent. was above 32° F. and of the maximum temperature curve only 17 per cent. was above 32° F. during the period January 1 to April 30.

PHYSICAL CONDITIONS OF LEAF SAP DURING CONVERSION

Some features of the differences in the cell organisation during winter and summer were described by us in a former paper[2]. These observations were extended during the next year, especial attention being paid to the physical conditions of the cell at the time of the spring change.

Readings were begun during early March and were continued until the end of April, the spring change having occurred about April 5. They are recorded in Table I. The sap after being extracted by the methods described in a former paper[2], was filtered, and the Δ and conductivity determined immediately after extraction by the Kohlrausch method. The viscosity of the sap was measured with an Ostwald viscosimeter and the conductivities corrected for viscosity.

Maximum and minimum daily temperatures (F.), January to April 1920, at Edmonton, Alberta.
——— Maximum.   ----- Minimum.
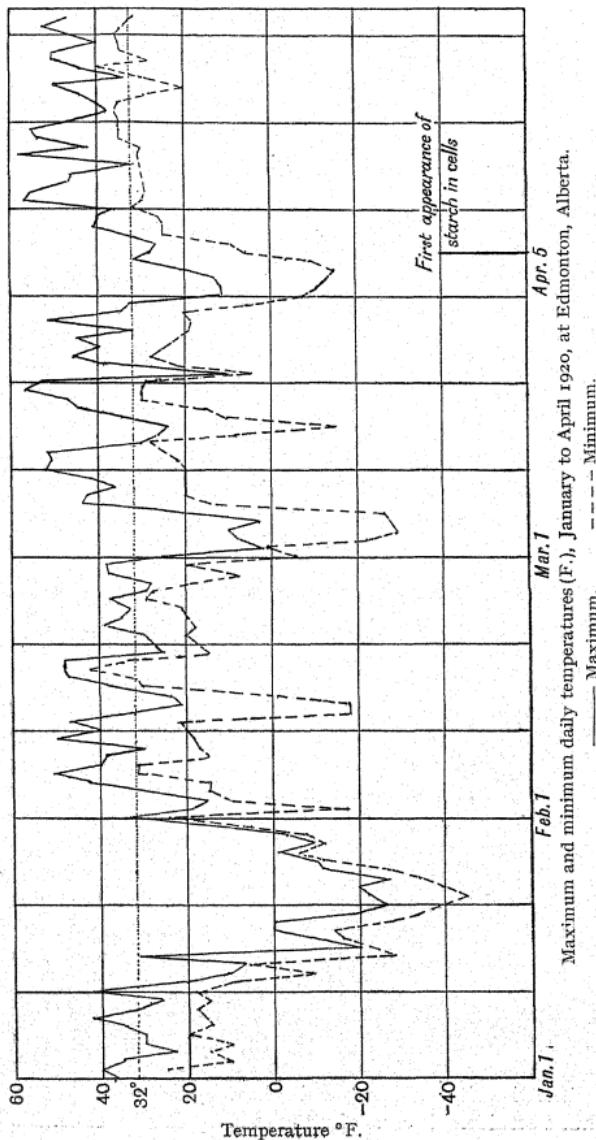
TABLE I

Date	Δ	P	Viscosity	Conductivity $\times 10^6$ corr. for visc.	Water content %	Condition of cells
Mar. 9	1·560	18·76	2·0	1006	55·0	Winter condition
,, 12	1·820	21·88	2·2	675	54·7	,, ,,
,, 15	1·701	20·45	3·0	1083	51·3	,, ,,
,, 22	1·725	20·74	3·0	1044	—	,, ,,
,, 27	1·858	22·34	2·48	897	—	,, ,,
April 1	1·830	22·00	3·0	1050	—	,, ,,
,, 6	1·777	21·36	2·5	867	53·1	Minute quantities of starch
,, 7	1·887	22·67	3·3	1072	49·3	,, ,, ,,
,, 13	1·781	21·66	2·76	816	48·7	,, ,, ,,
,, 17	1·797	21·59	3·30	917	—	Increased quantity of starch. Contents of cell still localised
,, 29	1·380	16·60	2·64	844	60·4	Larger amount of starch. Cells in summer condition
May 6	1·493	17·96	2·70	852	51·0	As on April 29

The atmospheric pressure P is the value corresponding to the observed Δ given in the tables published by Harris and Gortner[3].

As regards the osmotic pressures it will be observed that after maintaining a remarkable uniformity, a decided drop occurred at the time the cells began to pass into the summer condition. A marked drop in the total sugars in the sap was recorded in 1919 at this time[2], but a partial recovery both in osmotic pressure and sugar content appears to take place at the beginning of summer.

The water content of the tissues may be partly responsible for the change in pressure, but the evidence for this is not conclusive. The water content appears generally to diminish during late winter and rise again when photosynthesis and transpiration become active. But material fluctuations occur for which light and temperature may be responsible; for example, on April 7 and 13 the water content was 49·3 and 48·7 respectively. This was during a period of moderate warmth when the day temperature varied between 40° to 55° F., whilst the night temperature dropped to about 20° F. and the cells were already in the state of change from winter to summer condition. On April 29 the water content had risen to 60·4 per cent. This was during a warm period when both absorption from the soil and transpiration were exceedingly active. The drop to 51 per cent. on May 6 was probably due to excessive transpiration, as daily temperatures of 70° F. were then recorded, with a hot dry wind.

The viscosity of the sap varies from 2·5 to 4 at different periods. Thus a considerable correction has to be applied to the conductivity.

This was not done in a previous paper, neither was the viscosity of the sap considered by Dixon and Atkins in their earlier work (4).

<div align="center">CONDITIONS AFFECTING CONVERSION</div>

In 1919 the change from the winter condition in the organisation of the cells and the first appearance of starch began to take place on April 7. The stages in the gradual re-formation of the chloroplasts and their movement from the region of the nucleus and the appearance of starch, were described by us in a former paper (2).

One of the most suggestive features is the apparent want of connection between the first appearance of starch in outside material and the daily temperature curve. Examination of the graph shows that from March 29 to April 7 the maximum temperature never rose above freezing-point and on April 3 a minimum temperature was recorded of − 15° F., yet on the afternoon of April 5 the first trace of minute starch grains could be demonstrated with the use of iodine and chloral hydrate. Leaves had been examined daily for some two weeks previously, and more frequently as the suspected time of conversion approached, but no trace of starch could be demonstrated with the use of iodine and chloral hydrate; material outside still began to form starch at a certain date, although the temperature never rose above 32° F. and fell to − 15° at night.

The evidence strongly suggests a certain rhythmic change, independent, as regards its initiation, of external factors such as temperature. This is supported by an examination of coniferous material in the neighbourhood of Edmonton, at 2240 feet, and from altitudes up to 6500 feet in the Rocky Mountains. The date of the change from winter to summer condition in *Picea canadensis* was the same in trees grown at Edmonton, and at Banff at an altitude of 4600 feet. It was found that *Picea Engelmanni* (Parry) Engelm. from 6400 feet, *Abies lasiocarpa* (Hook.) Nutt. from 6400 feet, *Pinus Murrayana* Balf. from 4600 feet, *Pinus albicaulis* Engelm. from 6500 feet, all in the neighbourhood of Banff, showed the same changes in the reorganisation of the chloroplasts and the appearance of starch at the same date as white spruce material at Edmonton, where spring conditions are at least six weeks in advance of the sub-alpine zone in the mountains. But temperature evidently acts as an accelerator for it was not until April 29 that outside material showed as much starch as that obtained from material exposed to laboratory temperature for 24 hours on April 5. It is suggested that an activator,

such as a hormone, may be responsible for initiating the change and further work to test this hypothesis will be carried out.

Sinnott(5) has recorded the fact that change of temperature alone is quite insufficient to account for the seasonal change in food reserves and records that these changes occur in trees growing in the frostless area of the Gulf of Mexico.

Further it has been found that the position of the chlorophyll in the cell varies with the temperature. At moderate temperatures of about 0° F. the chlorophyll remains irregularly diffused in the cell, but showing a tendency to collect in the neighbourhood of the nucleus. At extremely low temperatures of − 20° F. or below, it becomes closely retracted round the nucleus, whatever position the latter may take up in the cell, to such an extent as completely to mask the nucleus. On a rise of temperature the chlorophyll. tends again to become more diffuse, although the change of position may take more than 24 hours.

The browning of the chlorophyll during the winter season is a very marked feature, and it is even noticeable in leaf sections under the microscope as well as in the foliage.

Experiments have been carried out to ascertain what part, if any, light plays in the conversion of the chlorophyll from the winter to the summer condition as well as the first appearance of starch in the spring. For this purpose a number of branches of Picea were covered during February and March with bags of closely-woven black material made in double thickness, the mouth of the bag being tied tightly round the branch. Trees were selected growing in different aspects, some exposed to sun and others in a shady position on the northward facing slopes of the Saskatchewan Valley. Some of these observations are recorded in Table II. The light-tight covering was placed on lateral branches of the same main branch used as a control, so that in all cases the experiment and control were side by side. Leaves were withdrawn for examination during February, March and April without the bags being removed.

No change took place either in experiment or in control until the critical date of April 5, when starch appeared both in leaves exposed to the light and those in darkness.

Both in laboratory experiments described in a former paper(2) and in field experiments, the first-formed starch in the spring is not the direct result of carbon assimilation, as it appears in the dark at the same time and in the same amount as in leaves in light.

Complete reorganisation of the chloroplasts in the field experi-

ments did not take place until the beginning of May and at this period no distinction could be recognised between the experiments and controls. In both cases the chlorophyll had changed from the winter brownish yellow colour to the normal vivid green and the chloroplasts had become completely reorganised with normal grouping in the cells.

The regularity of these changes regardless of the influence of light and the first appearance of starch at temperatures below the freezing-point are interesting features.

TABLE II

Condition of the chloroplasts and the appearance of starch in light and darkness

Date 1920	Location in	Material	Condition of the chloroplasts	Starch	Oil
6. iv.	Shade	Covered Mar. 23	As in winter	Small amount	Abundant
6. iv.	,,	,, ,, ,,	,, ,,	,, ,,	,,
6. iv.	,,	In light	,, ,,	,, ,,	,,
12. iv.	,,	Covered Mar. 23	Mostly distinct, frequently localised around nucleus	Abundant with chloral hydrate and iodine	,,
12. iv.	,,	In light	As in covered material	Abundant with iodine	,,
12. iv.	Sun	Covered Mar. 23	Faint, but evenly distributed, generally distinct	Abundant with chloral hydrate and iodine	,,
12. iv.	,,	In light	Rather less organised than in preceding	Abundant with iodine	,,
4. v.	Shade	Covered Mar. 23	Chloroplasts fully formed	Trace with iodine, abundant with chloral hydrate and iodine	,,
4. v.	,,	,, Feb. 1	,, ,, ,,	Abundant with iodine and chloral hydrate	,,
4. v.	,,	In light	,, ,, ,,	Abundant with iodine	,,
25. v.	,,	Covered Feb. 10	,, ,, ,,	Small amount with chloral hydrate and iodine	,,

Observations have been carried out every year since 1920–1 and the changes recorded in a former paper(2) have been substantiated and further facts brought to light. The change from summer to winter condition seems to be very gradual. On October 28 the contents of the mesophyll cells were tending to become localised. The chloroplasts, still definite in outline, were crowded round the nucleus in which the chromatin was very distinct, indicating the progress of rapid chemical changes in the cell. Cells examined during November and December showed the chloroplasts becoming less distinct in outline until in many cells no trace of their form could be distinguished. The extreme winter condition was observed in cells examined on January 26. At this time the green colour was confined to a small portion of the cell, while the major portion was

occupied by fat. A variation was observed in different cells, some showing a greater disorganisation of the chloroplasts than others, but a definite outline was not observed at any period when exposed to low temperature.

SUMMARY

1. Osmotic pressures, electrical conductivities and viscosities of the sap and the condition of the leaf cells of *Picea canadensis* have been determined through the period of change from winter to summer condition.

2. There is a rapid drop in the osmotic pressure from the winter level of over 20 atmospheres to 16 or 17 atmospheres at the date when the leaf cells pass from the winter to the summer condition.

3. The initiation of conversion from the winter to the summer condition is independent of a rise of temperature, starch having first appeared on April 5, 1920, after a period of six days, when the maximum temperature remained below 32° F. and minimum temperatures of − 14° F. were experienced.

4. Material of other conifers from an elevation of 6500 feet in the Rocky Mountains shows conversion at approximately the same date as that from the Edmonton district at 2240 feet.

5. Reorganisation of the chloroplast, migration of the chloroplast from the nucleus, and changes in the chemistry of the pigments, take place in darkness at the same time and to the same extent as in light.

6. The first appearance of starch in the cells takes place in darkness as well as light and at temperatures below freezing-point.

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PRIMULA VULGARIS VAR. *CAULESCENS*

By MILLER CHRISTY, F.L.S.

IN a recent paper on the British Hybrid Primulas, I stated[1] that the "Common," "False," or "Hybrid" Oxlip (*P. veris* × *vulgaris*) had been known, among other names, as *P. vulgaris* var. *caulescens*[2].

This statement was correct so far as it went, but I should have added that it was correct only because the hybrid in question has been confused persistently with another plant—similar, but quite distinct—to which the name in question rightly belongs. That other plant is a *variety* (*not a hybrid*) of the Common Primrose (*P. vulgaris*) and is met with occasionally wherever that plant occurs.

The resemblance between the two plants is sufficiently close to account for their having been frequently confused. I myself had been for years quite familiar with both before I clearly realised that they were essentially distinct. Yet the points of difference between them, though slight, are, when once recognised, sufficiently marked to enable anyone who has the two plants before him in the *fresh* state to discriminate between them with little or no difficulty[3].

The variety (to which, as stated, the name *P. vulgaris* var. *caulescens* rightly belongs) may be recognised by the fact that, though its flowers are borne in umbels, they are in all other respects *of the pure Primrose type*, having the tight-fitting calyx, the flat corolla-limb, and the pale yellow corolla, which are characteristic of that plant. Another feature of the variety is that its umbel is often irregular (some of the pedicels branching from the side of the pe-duncle, near its top, instead of all together from its top), and is always more or less lax and straggling, with a short peduncle and long pedicels.

The hybrid (to which, as stated, the name *P. vulgaris* var.

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[1] See *New Phytologist*, **21**, p. 299, 1922.

[2] The name is that of Koch (*Synops. Fl. Germ. et Helvet.* p. 587, 1837).

[3] This cannot be said of them, however, when in the dried state. Indeed, I doubt if anyone could discriminate between them with certainty when dried; for their leaf-characters are too indefinite to afford reliable guidance, even when fresh, and their distinctive floral characters, including the colour, are almost completely obliterated by drying.

caulescens is often applied in error) is the Common or Hybrid Oxlip (*P. veris* × *vulgaris*), which, though seldom abundant, is generally met with sporadically wherever its two parents, the Cowslip and the Primrose, grow in proximity, as they do almost everywhere[1]. Its characteristic features are less easy to point out, inasmuch as (owing to its hybrid origin) these are very variable. Thus, its flowers, though generally produced in umbels, are frequently single (*i.e.* acaulescent), like those of its primrose parent, and both forms of inflorescence often appear on the same plant. Even when the flowers are produced in umbels, those umbels are generally more or less lax and straggling, showing little trace of the well-marked one-sided droop of the umbel of its cowslip parent. More reliable features are to be found in the actual flowers, which are always intermediate in character between those of the plant's two parent species. Thus, the widely-inflated calyx of the cowslip is generally, if not always, more or less apparent. Again, the sharp ridge or fold, with notches in it, in the throat of the corolla-tube (which is always apparent in the flowers of the cowslip) is, I believe, always present. Their colour is a fairly-good criterion; for the flowers of the hybrid are never, I believe, of the pale yellow colour of the primrose, the deeper yellow of the cowslip being always more or less dominant.

That pure (unhybridised) plants of the primrose bearing their flowers in umbels really do occur in the natural state was recognised as long ago as 1792, when Thomas Martyn, F.R.S., wrote[2]: "We have sometimes met with a primrose in a wild state pushing up a scape which sustained several flowers differing in no respect from the ordinary sort, except in this circumstance." Since that time, the occurrence of the caulescent variety of the primrose and its distinctness from the hybrid between that plant and the cowslip has been fully recognised by not a few English botanical writers, but overlooked by as many others. The distinctness of the two plants has been more generally recognised by French botanists, though many of them also have confused the two. Thus, Godron, after mentioning[3] that he has met with the variety in France, "quoique très rarement," adds: "nous distinguons positivement le *Primula variabilis* [*i.e. P. veris* × *vulgaris*] de la forme caulescente du *Primula grandiflora* [= *P. vulgaris*]." Again, Mons. E. Legué, after speaking of the hybrid[4], says: "il existe bien une forme caulescente

[1] See *New Phytologist*, **21**, p. 299, 1922.
[2] *Fl. Rustica*, p. 62, 1792.
[3] *Bull. Soc. Bot. de France*, **10**, pp. 179–180, 1863.
[4] *Idem.* **29**, p. 133, 1882.

du *P. vulgaris*,, mais elle est très rare et je l'ai toujours vue à la hampe très courte et les pédicelles très allongués[1]."

One strong proof that the caulescent or umbellate variety of the primrose is not due to any crossing with the umbellate cowslip is to be found in the fact that the variety occurs in districts from which the cowslip is absent; so that there can be no possibility of hybridisation.

Thus, my friend the late Mr E. A. Fitch, of Maldon, Essex, when sending me, in April, 1905, specimens of the caulescent primrose which he had found in Hazeleigh Wood, near that town, remarked that their umbellate flowers could not be ascribed to hybridisation with the cowslip, inasmuch as no cowslips were growing (he believed) nearer than a certain Paigle Mead, in Woodham Walter parish, quite two miles and a half distant—indeed (he added), as far as he knew, there were no cowslips in the whole Essex Hundred of Dengey, the soil of which, being almost exclusively London Clay, lacks the lime which the calciphile cowslip demands. Similar evidence is forthcoming from France. Mons. Aug. le Jolis, of Cherbourg, says[2] that he has never met with the hybrid oxlip (*P. veris* × *vulgaris*) in his neighbourhood[3], from which both cowslip and (true) oxlip are absent, but he says he has met with the caulescent variety of the primrose at Urville and at Octeville, adding "j'y ai observé souvent des formes où la hampe était accompagnée de pédoncules radicaux uniflores." Again Mons. E. Lebel has described[4] specimens of the variety which grows with primroses of the normal form among mowing-grass on the slope of a sea-cliff at Lestre

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[1] It should be noted that the caulescent variety of the primrose described above is usually much nearer to, and more difficult to distinguish from, the hybrid between the oxlip and the primrose (*P. elatior* × *vulgaris*; see *ante*, p. 234) than from the hybrid between the cowslip and the primrose (*P. veris* × *vulgaris*). Indeed, the two are scarcely to be distinguished, except by a critical botanist who has seen both actually growing. The oxlip × primrose hybrid, like the cowslip × primrose hybrid, bears its flowers both singly and in umbels (both forms of inflorescence frequently appearing on the same root, as might be expected in view of the plant's parentage), and these flowers are always of a pale yellow (the colour of both its parents). Its most pronounced points of difference from the caulescent primrose are that its umbels are even more loose and straggling; the peduncle is usually very short; and both it and the pedicels are very hirsute. The oxlip × primrose hybrid is, however, very scarce in Britain, being met with only where the two parent species come into contact on the extreme margin of the limited area in the eastern counties to which the oxlip is confined.

[2] See *Mém. Soc. Sci. Nat. de Cherbourg*, 7, p. 312, 1860; also *Bull. Soc. Bot. de France*, 8, p. 629, 1861.

[3] He speaks of it as *P. variabilis* Goupil, its usual name in France.

[4] *Bull. Soc. Bot. de France*, 11, pp. 88–91, 1864.

(Manche), a locality he has known and visited annually for seventeen years. During that time, the plant, which bears its flowers in loose umbels and occasionally singly, has (he says) maintained its character. That the plant cannot be a hybrid is proved by the fact that Mons. Lebel has never seen a cowslip in the vicinity, the nearest locality for it being (he believes) 18 kilometres distant.

That an ordinary pure-bred acaulescent (single-flowered) primrose plant should produce caulescent (umbellate) flowers must be due, of course, to some special cause, and that cause appears to be usually some sudden and disturbing change in the plant's conditions of growth or abnormality in its environment. As to this, the late Rev. E. A. Woodruffe-Peacock wrote[1]:

I have come to the conclusion, from experiments [? observations] in woods and gardens, that the *caulescens* form of *P. vulgaris* has nothing at all to do with hybridity, but is merely a forced abnormal state. [I have observed it, he goes on] only where sudden change has taken place, as a fall of trees in woodland or a sudden change in garden cultivation.

I cannot recollect having ever observed that the cutting down of a wood containing primroses induces them to produce caulescent flowers, though I have seen much in Essex of the effects of that process in stimulating the growth and free flowering of the plants; but it seems likely that the change in question may be thus produced.

That the primrose may be induced by cultivation to produce caulescent (umbellate) flowers seems certain—is, indeed, commonly seen. As long ago as 1790, William Curtis wrote of the primrose[2] that "the plant, when cultivated, will sometimes throw up a stalk similar to that of the Polyanthus; and of this my very good friend Dr Buxton, of Greenwich, has favoured me with a striking instance."

Mr Charles Nicholson writes[3]:

The umbellate form of the primrose (var. *caulescens*) is simply an example of the lengthening of the peduncle, which is...only developed as such occasionally.

I believe this phenomenon (the caulescent truss) to be commoner in some seasons than others, and it is probably regulated by weather conditions. At any rate, it is very uncertain in its appearance.

I have found that plants of this form, when transplanted to the garden, have reverted to the ordinary form in the next year. I have been told, on the other hand, of ordinary primrose plants having

[1] In a letter, dated October 14, 1910, to Mr Charles Nicholson.
[2] *Fl. Londin.* 6, p. 16, ? 1790 (see also Withering, *Brit. Plants*, 2, p. 233, 1796).
[3] *Gardener's Chron.* June 18, 1921, p. 301.

developed the caulescent form in a garden in one season only, there being no cowslips or oxlips anywhere near.

Prof. J. W. Heslop Harrison, of Newcastle-on-Tyne, has favoured me with the facts of a case in which growth in an extremely-wet situation was, apparently, the actuating cause which led certain primrose plants to produce caulescent flowers. In the north of England, he says, he

once found several plants with very-pronounced stalks growing in a waterfall. These, when removed to the garden, promptly reverted to the ordinary form and remain so now. I cannot [he adds] conceive of these plants being hybrids. The strange habitat and other points are against it.

It is, I think, easy to understand that an excess of moisture might induce the primrose, a marked lover of moisture, to develop its latent peduncle; and the fact that the plants in question reverted to the normal (acaulescent) type of inflorescence when removed to a drier situation seems strongly to support this view.

Further, there seems good reason to believe that this tendency of the primrose to produce caulescent (umbellate) flowers in cultivation is, for some reason, specially marked in the western parts of England and Wales. Thus, Lady Thistleton-Dyer writes[1]: "Nearly all the garden primroses have a tendency here to become polyanthus[2]. Even the blue primrose, which is from a plant divided up, has few single flowers now." Again, in Pembrokeshire, where the red variety of the primrose abounds and is frequently dug up by cottagers for removal to their gardens, Mr J. E. Arnett, of Tenby, informs me he has been told that plants so removed "grow like a polyanthus in two or three years." There is other evidence to the same effect.

It is, in any case, in no way remarkable that the primrose should be very ready, under stimulation of any kind, to produce its flowers in umbels; for there can be no doubt that this is no more than a reversion to an ancestral form of inflorescence. The plant belongs to a large and widespread genus, comprising at least 250 described species, practically all of which bear their flowers in umbels. Indeed, it is easy to satisfy oneself that the flowers of the primrose, though apparently borne singly, *are in reality borne in umbels*; for examination of any plant when in full flower will show that the pedicels on which its flowers are borne all spring from one or more definite

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[1] In a letter, dated May 15, 1922, to Mr Charles Nicholson.
[2] Here, clearly, the word is used adjectivally in its literal sense of many-flowered: not as the name of the particular many-flowered primula commonly known as "the polyanthus."

points on the rootstock (*i.e.* the short compact stem from which leaves and flowers arise), at or slightly below the level of the ground[1]. Often as many as eight or ten pedicels spring from one of these points, each having at its base a long narrow lanceolate bract, 10–12 mm. long and about 1 mm. broad at the base. It is, therefore, correct to say that the flowers of the primrose are borne normally in umbels—that is, in *sessile umbels*, the scape or peduncle having been suppressed. The umbel is complete, even to the bract which, in the other umbellate species of the genus, appears at the top of the peduncle, on the outer side of the base of each pedicel.

The caulescent or umbellate variety of the primrose is, therefore, merely a variety in which the plant has developed the peduncle, usually suppressed, thereby reverting to an earlier form of inflorescence. But why a tendency to this reversion should be more pronounced in one district than in another (as seems to be the case) is by no means easy to explain.

P.S.—Since the foregoing was written, Prof. J. W. Heslop Harrison has informed me that, in the north of England, he has sometimes met with a plant which, though distinct in origin from the caulescent variety of the primrose, resembles that plant so closely that it could probably not be distinguished from it by even the most careful external examination—and this is a back-cross between the hybrid oxlip and the primrose (namely (*P. veris* × *vulgaris*) × *P. vulgaris*).

I have never seen (or, at all events, have never recognised) this hybrid in the south of England, where, indeed, I doubt its occurrence; but Prof. Heslop Harrison has proved its existence in his district by means of cytological tests[2]. He has also produced it by artificial

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[1] Curtis' denial (*Fl. Londin.* 6, p. 16, ? 1790) that this is the case must have been due to an error of observation.

[2] In the south, the ordinary hybrid oxlip (*P. veris* × *vulgaris*), though it occurs wherever its two parent species grow in proximity, is always scarce and sporadic (see *ante*, 21, p. 299); but, in the north, it occurs in places quite abundantly. Thus, the late J. G. Baker writes of its two parents (see *Journ. Roy. Hort. Soc.* 7, p. 212) that "you cannot go into any field in the north of England without seeing that they do hybridise most fully [? freely]." In Northumberland and Durham, Prof. Heslop Harrison informs me that the hybrid oxlip is, in places, extremely abundant, especially on the Magnesian Limestone on the coast, whence "countless" plants may be obtained. The reason for the exceptional local abundance of this hybrid is, he says, the prevalence in early spring of cold north-east winds, which greatly retard the flowering of the primrose, especially on the coast, causing the flowering-times of it and the cowslip to synchronise much more nearly than elsewhere: hence more frequent hybridisation between them.

pollination in the garden. Those plants he has examined had all developed some giant pollen-grains—one of the recognised indications of hybridity.

In regard to the flower of this back-cross hybrid: Prof. Heslop Harrison says that it presents points of difference from that of the caulescent variety of the primrose, but that, having never had flowers of the two plants in hand together for purposes of comparison, he is unable to state exactly in what respects they differ. His impression is, however, that, in the caulescent variety, the individual flowers are slimmer than those of the back-cross hybrid and are borne on longer stalks.

But the fact that this back-cross hybrid (which probably does not occur everywhere) undoubtedly exists in no way alters the status of the caulescent variety of the primrose dealt with above, though it certainly adds to the difficulty in distinguishing it.

PERMEABILITY

By WALTER STILES

CHAPTER XIV (_continued_)

THEORIES OF CELL PERMEABILITY (_continued_)

THE VISCOSITY THEORY

It has been suggested by Spaeth (1916) that the permeability of the plasma-membrane is determined by its viscosity, which is itself determined by the degree of dispersion of the colloids contained in it. On this view increased permeability is to be regarded as due to increase in rate of diffusion consequent on decrease in viscosity (cf. Chapter IV).

This theory must be regarded as a solution theory of permeability, as it regards permeability as determined by diffusion through the plasma-membrane; it does not, however, define the chemical composition of the plasma-membrane and so might be applicable to any solution theory of permeability.

A consideration of the state of affairs postulated by the viscosity theory shows how the ultrafiltration and solution theories can be

brought into harmony, for if the protoplasmic layer determining permeability is a viscous sol or gel, the rate of penetration of a substance through the gel will, according to the formula of Einstein, Sutherland and von Smolukowski, depend on the size of the molecules as well as on the viscosity, and it is reasonable to suppose that molecular aggregates above a certain size may not be able to diffuse through the spaces between the particles of the disperse phase of the gel. Such considerations lead on to the whole question of the nature of solution into which we cannot enter here.

A number of objections to Spaeth's theory have been raised by Osterhout (1916 a), chiefly based on the fact that changes in viscosity of *Laminaria* thallus produced by placing this tissue in mixtures of sodium and calcium chlorides do not run parallel with changes in electrical resistance. What appears to the writer to be a very strong argument against the viscosity theory is that the changes in viscosity of a gel are scarcely great enough to account for any great differences in permeability (cf. Stiles and Adair, 1921).

The theory of antagonism advanced by Osterhout (1916 b) assumes, as we have seen, that the permeability is inversely proportional to the electrical resistance of a cell, and that the resistance is proportional to the quantity and the thickness of a particular substance in the protoplasm. While this theory would not be incompatible with a viscosity theory, inasmuch as the resistant substance might be a viscous one through which substances entering the cell had to diffuse, there are other possibilities. Thus, the substance to which the cell owed its resistance might be impermeable to entering substances, so that with increase in the relative amount of the impermeable substance the proportion of the protoplasm (or plasma-membrane) through which material could enter would be smaller, and hence permeability would be reduced. In such a case the question of the correctness of a sieve or solution theory is left untouched.

THE PHASE INVERSION HYPOTHESIS

To account for the supposed action of various electrolytes on cell permeability, Clowes (1916 a, b, c; 1917 a, b, c) also makes use of the colloidal character of protoplasm. As protoplasm can be regarded as an emulsoid colloidal system, there will be a continuous phase of one composition (dispersion medium) through which is dispersed at least one other phase, the particles of which are not continuous. Clowes supposes changes in permeability are brought about by the continuous and discontinuous phases changing places, as is supposed

to happen when a gelatin or agar sol sets to a gel. In this way a substance which could not diffuse through the continuous phase owing to insolubility in it might be able to diffuse through the discontinuous phase but would not be able to enter the cell because of the discontinuity of the disperse phase. With inversion of the phases, penetration would then take place at once. The hypothesis would be equally applicable to a solution or a chemical combination theory of permeability.

Clowes produces support for his theory from experiments on the effects of sodium hydroxide and calcium chloride in producing phase inversion in emulsion systems of olive oil and water, which are compared with the effects of these substances on protoplasmic permeability.

Free (1918) has subjected this hypothesis to some criticism. He points out very rightly that such a hypothesis suggests that permeability changes would be sudden, and even if it were assumed that the protoplasm (or plasma-membrane) were in a state of mobile equilibrium so that parts of it were in one state and other parts in the inverse condition, with frequent alteration in the relative quantity of the two parts which is the state of affairs Clowes appears to suggest, the balance would in all probability be very easily upset so that the whole of the protoplasm would go to one or other of the conditions.

A further weakness of the hypothesis is the absence of any direct experimental evidence in its support, for the bearing of experiments with olive oil-water emulsions on cell permeability are, as Free suggests, rather dubious, as such emulsions are not generally present in cells.

LLOYD AND FREE'S COLLOIDAL HYPOTHESIS

The hypothesis of protoplasmic permeability put forward by Free (1918) and earlier suggested by Lloyd (1915) bears certain points of resemblance to the two hypotheses just discussed. These writers also start out from the supposition that the protoplasm consists of an emulsoid colloidal system. It is further supposed that two (at least) of the liquid phases of protoplasm differ importantly only in the proportions of water which they contain. Changes in permeability are supposed to be due to changes in the distribution of water between the continuous phase (dispersion medium) and the discontinuous (disperse) phase. When the globules of the disperse phase are large the spaces between them will be small, and *vice-versa*, and a substance diffusing through the continuous phase will travel faster or slower according to the dimensions of the spaces. Solubility in the disperse phase alone, and not in the dispersion medium, would, of course, not permit the penetration of a substance.

A powerful argument against this hypothesis, as against the viscosity hypothesis of Spaeth, which it may indeed be regarded as including, is that actually such changes in the relative quantities of water in disperse phase and dispersion medium do not appear to produce any very great change in the rate of diffusion of substances through a colloidal system. What appears to be essentially a modification and elaboration of this hypothesis will be mentioned at the end of this chapter under the head of electrical theories.

TRÖNDLE'S THEORY OF PROTOPLASMIC IRRITATION

It has already been noted that from data obtained by a deplasmolytic method Tröndle concluded that the rate of intake of a salt is at first independent of the concentration of the salt, and that after about the first ten minutes of exposure to the salt the rate of intake falls off according to a logarithmic relation. From this Tröndle concludes that salt intake takes place by the salt irritating the protoplasm which responds by conveying the salt into the vacuole. After the first ten minutes the protoplasm exhibits fatigue, and salt intake falls off according to Weber's law.

Tröndle (1920) considered that he had obtained confirmatory evidence of the correctness of this theory from the fact that if cells are treated with a dilute solution of a narcotic before immersion in the salt solution, the intake of the salt is retarded or even completely inhibited. The narcotic is supposed to prevent the participation of the protoplasm in the absorption process and in consequence the salt is not taken up. On the other hand, when the protoplasm is rendered inactive by preliminary treatment with dilute acid (0.01 N oxalic acid or 0.005 N hydrochloric acid) for five minutes, according to Tröndle the intake of salt is then proportional to the concentration of the salt, thus obeying Fick's law, although his figures do not appear to support this assumed relation.

In an earlier chapter reasons have been given for not accepting the conclusions of Tröndle with regard to the course of absorption. But even if the results were acceptable, the only legitimate conclusion that could be drawn from them with regard to the mechanism of salt absorption would be that the passage of salt, during the first ten minutes, did not take place by simple diffusion in a solvent, so that a solution theory of permeability to salts would be inadmissible. To conclude that a phenomenon of stimulation is in question simply because after ten minutes the intake of salt falls off with time according to

a logarithmic relation, appears to be completely unwarranted. The same result would be expected, if, after the first ten minutes, the intake of salt were a simple diffusion process governed by Fick's law. Clearly no part of Tröndle's theory can be accepted.

THE ADHESION THEORY

J. Traube, in a long series of papers (1904 *a, b, c*, 1908, 1910 *a, b*, 1911, 1913 *a, b, c*, 1919), has put forward a theory of permeability, the essential feature of which is that the capacity of a substance to diffuse into a cell depends on the extent to which it lowers the surface tension of water in contact with air. In the papers of Traube cited, the evidence for this will be found. The arguments against the adhesion theory are two, and they are fatal. In the first place the measurements of surface tension made by Traube are against air, whereas what is actually concerned is the surface tension of the solution (that in the cell wall in the case of plants) against protoplasm. There appears to be no direct relation between the surface tension of a liquid against air and its surface tension against another liquid, and there is no known way of calculating it. In the second place, as pointed out by Collander (1921), the measurements of Höber (1914, *b*) and, indeed, of Traube and Köhler (1915), show that whereas a great difference exists between acid and basic dyes in regard to their absorption by plant cells, there is no constant distinguishing difference between the two groups as regards the surface tension of solutions of them against air. Under these circumstances it does not appear worth while to discuss the theory in any detail.

The extension of Traube's theory in which the adhesion of the constituents of the protoplasm is also supposed to influence the permeability, must, as pointed out by Collander, if it is to have a definite meaning, attribute permeability either to the solvent or adsorptive properties of the protoplasm. In the first case it becomes a solution theory, in the latter an adsorption theory as described below.

CHEMICAL COMBINATION AND ADSORPTION THEORIES

A number of writers have held that some substances enter living cells by means of adsorption or chemical reactions. The substance combines chemically with, or is adsorbed by, a constituent of the protoplasm (or plasma-membrane). This disturbs the equilibrium between different cell constituents, with the result that the chemical or adsorption compound breaks down again and the substance is

released on the other side of the plasma-membrane or combines or is adsorbed by some other cell constituent according to the molecular affinities of the various constituents.

Pauli (1904) appeared to consider that salts were absorbed by their combining chemically in this way with the plasma-membrane, and the same opinion was held by Traube-Mengarini and Scala (1909) working with algæ and protozoa. The work of Szücs (1910, 1911, 1912, 1913) appears rather to suggest adsorption as responsible for the intake, and Pantanelli (1915 *a, b, c*, 1918) also lays emphasis on the importance of adsorption.

It is to be observed that this theory can be applied equally well to a cell surrounded by a plasma-membrane and to one in which the protoplasm is approximately homogeneous, and indeed Moore (1921) and his co-workers (Moore and Roaf, 1908; Moore, Roaf and Webster, 1912), who deny the existence of plasma-membranes, are supporters of this view.

Szücs (1912) appears to think a combination of the lipoid theory and adsorption theory is possible, the intake of dyes and narcotics being explained by their solubility in fatty substances, while salts enter by adsorption or chemical combination. The relation between external concentration and the position of the equilibrium attained in the absorption of salts by some plant cells can be used in support of this view, but it must be mentioned that at least some dyes appear to follow the same rule (Redfern, 1922 *b*).

<div align="center">ELECTRICAL THEORIES</div>

The great difference between acid and basic dyes in regard to their penetration into cells, and the assumed influence of acids and alkalies in influencing the intake of these dyes, has suggested the possibility that the electric charge on the plasma-membrane may be of importance in determining whether particular ions are absorbed. The theories propounded (*e.g.* Girard, 1914 *a*, 1919 *a, b*) are at present mostly vague, and the foundation of fact on which they rest insecure (cf. Collander, 1921) so that a discussion of such theories in the present state of our knowledge would be scarcely profitable. It is not at all clear, moreover, whether such theories in the end do not resolve themselves into special cases of the adsorption theory of permeability.

A theory of cell permeability combining an electrical theory with a colloidal theory closely resembling that of Lloyd and Free, has recently been propounded by Miss Haynes (1921), who assumes that

the plasma-membrane is a gel of which the more solid phase consists principally of amphoteric emulsoid colloids and the more liquid phase of a buffer mixture. At the iso-electric point the continuous phase will be in a state of minimum hydration and so will occupy a minimum volume, and also will be without charge. It will in consequence be most permeable in this condition. Above the iso-electric point the membrane will possess a positive charge and so will repel kations, while below the iso-electric point the membrane will have a negative charge and will therefore repel anions. Only in the immediate neighbourhood of the iso-electric will such a membrane be permeable to ions. Modification of permeability, brought about by the addition of various substances, will, on this theory, be due to changes produced in the reaction of the buffer mixture of the more liquid phase of the protoplasmic membrane, and the phenomena of antagonism can be similarly explained.

CHAPTER XV

CONCLUDING REMARKS

IN this review of our present knowledge of the permeability of plant cells and related phenomena, I have attempted to bring together and correlate the work done by many different workers on different lines and by the use of different methods. Anyone who takes the trouble to read the literature of the subject can scarcely fail to be impressed by the isolation in thought of the majority of workers in this field. That this neglect by the individual investigator of the work of others in the same subject is not a satisfactory condition of affairs a single example will suffice to make clear. It had become evident from the work of Nathansohn in 1903 that it is possible that the two ions of a salt are not absorbed in equivalent quantities by plant cells, and that the position of the equilibrium attained in the absorption of these ions is not one of equality of concentration outside and inside the cell. If these possibilities are facts, as they have now been shown to be, then plasmolytic methods of measuring salt intake, in which the rise of osmotic concentration of the cell sap is taken as a measure of the quantity of salt absorbed, cannot give results that are beyond question. Nevertheless, during the last five years, the results of quite a number of investigations with the use of plasmolytic methods have been recorded without the authors questioning

in the slightest the soundness of the method or of their conclusions.
Examples of this kind can be multiplied without difficulty.

It is hoped that the correlation of work along many different lines
attempted in this account of permeability will make it easier for the
student to grasp the present position of our knowledge, and also
make it easier for the investigator to avoid pursuing a line of attack
which acquaintance with other work in the same field would show him
could not further the advance of knowledge satisfactorily.

From the review attempted in the preceding chapters it should
be very evident how imperfect at present is our knowledge of the
permeability of plant cells, and, moreover, how very doubtful is the
significance of much of the experimental data on which our knowledge
rests. It cannot then be surprising if a review of the current theories
of cell permeability should lead to the conclusion that overwhelming
evidence in favour of any one of them is not forthcoming. To this
conclusion we are certainly led, and, indeed, it would seem rather
unlikely in any case that all substances should enter or be excluded
from the cell on account of the same mechanism, and ultrafiltration,
solubility in lipoid substances and other constituents of the proto-
plasm, adsorption and other surface effects, and chemical combination
may all play a part in determining whether any particular substance
is absorbed or not by the living cell. One point to be noticed in
particular is that with all these theories of cell permeability the
influence of the cell wall is usually neglected. Yet it seems to me,
especially in view of the work on semi-permeable cell walls dealt with
in an earlier chapter, and that of Hansteen-Cranner on the consti-
tution of the cell wall, that this neglect is not wholly justifiable, and
that to assume that the cell wall acts in no other way than as a dead
porous envelope is scarcely in accord with the facts.

In conclusion it should be emphasized how overloaded the whole
subject of permeability is with theories, and with observations based
on the assumption of the correctness of unproved theories. For this
reason many of the conclusions drawn from their observations by
various investigators are valueless. Not even the membrane theory
of the cell, and the simple view of the plant cell as an osmotic cell
surrounded by an elastic envelope, are really proved, although the
latter, within limits, has formed a good working hypothesis in regard
to the water relations of the cell. It clearly breaks down when used
to interpret the relation of living cells to dissolved substances. The
correctness of the theory of a semi-permeable membrane surrounding
the protoplasm is also dubious (cf. Davidson, 1916).

While the propounding of theories will continue to satisfy the minds of some, yet it cannot be too strongly emphasized that what are wanted to lay the foundations of a proper understanding of the phenomena of permeability· in plants are facts, and particularly quantitative data. When these are abundant where they are now scanty we may be able to formulate the laws governing the interchange of substances between the cell and its surroundings, and so be in a much better position for understanding not merely the mechanism of cell permeability, but also the life of the plant as a whole.

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THE SUPPOSED REVERSAL OF GEOTROPIC RESPONSE

By F. C. NEWCOMBE

BETWEEN two and three years ago there appeared a series of four papers in the *New Phytologist* by Small(4), Small and Rea(6), Small and Lynn(5), and Lynn(3) in which the authors offered experimental evidence for the reversal of geotropic response in the stems and roots of seedlings of a number of species of plants. The whole work was undertaken for the purpose of securing evidence for Small's so-called hydrion differentiation theory of geotropism. With the theory itself the present paper has no concern. The hydrion theory has been criticised by Blackman(1) and by Snow(7). The experimental data, however, are of such a character as to need consideration in themselves. Lack of time prevented the present writer from an attempt at verification of the results of Small and his followers till a year ago.

Small, for the support of his hydrion theory, starts with the assumption that the protoplasm of stems active in initiating the changes in a geotropic response is alkaline in reaction, while the corresponding protoplasm of roots is acid. This is a foundation stone in the structure of the hydrion theory, and is used to account for the difference in behaviour of stem and root to gravitation.

If now, by any means, the acid and alkaline reaction of the protoplasm in root and stem respectively could be reversed, the direction of geotropic response should reverse also. So reasons Small. To change the reaction of the protoplasm of the root from acid to alkaline, Small, after trying other methods, selected the following: A piece of flat cork was partially immersed in dilute ammonium hydrate for a few days, then allowed to stand for a month under a belljar with several changes of air. At the end of this period, the air under the belljar would still turn red litmus blue, but was not strong enough to kill the seedlings of *Vicia faba* and *Zea mais* pinned to the cork. Many roots turned up; none grew downward. Controls were used under other belljars which behaved in the usual way. To accompany the text, a plate and several text-figures are shown. Passing over the objectionable method of pinning the seedlings through the cotyledons or endosperm, it is to be regretted that one

must say that neither the figures in the plate nor those in the text can be accepted as lending evidence for the reversal of response. All five figures in the text, except number 4, show the roots shrivelled and distorted to such a degree that one used to the behaviour of roots must regard the figures as worthless for demonstration. Root number 4 in the plate is a fairly normally appearing root of *Zea mais*, but, unfortunately for the assumption of the reversal of geotropism, it is growing almost vertically downward.

In the three text-figures of *Vicia faba* roots, one root is so distorted as to exclude it from consideration. The placing of the other two figures, with the absence of any textual explanation of their peculiar form and appearance, prevents the reader from using them as an aid in forming his judgment. The three main roots of *Vicia faba*, just referred to, have growing from them 14 secondary roots from 1 to 25 mm. in length. Of these, only three can be counted as showing upward curvatures. The author speaks of fungi growing on his seedlings after two or three weeks. One must wonder why cultures of seedlings in damp chambers should be continued for so long a time; since the time of Sachs it has been published again and again, and it is the common experience of experimenters, that the roots of seedlings growing in damp chambers become distorted or unhealthy or die in less than two or three weeks.

In another series of experiments, Small grew seedlings in acetic acid vapour in damp chambers to see whether the stem would not show positive geotropism. He found that seedlings of *Zea mais* would grow fairly well over 1 per cent. of acetic acid. Of the 17 maize coleoptiles shown in the illustration, 13 bend downward 15 to 90°. The author states that the coleoptiles were about 25 mm. long when the seedlings were placed in the acid vapour. As in the illustrations the coleoptiles are shown with about 25 mm. length, it must be that the downward curves were made within a few hours of the introduction of the seedlings into the damp chamber, unless growth was very much retarded. Here again the reader must wish that the author had given greater detail in the text. With some reservation because of the lack of detail, it may be said that the figures of the coleoptiles shown support Small's claim of reversal of response.

The series of experiments by Small and Rea was carried out with the notion of causing the stems of seedlings to change from their assumed alkaline reaction to acid by smearing the shoots with vaseline, thus preventing the escape of the carbon dioxide of respiration. The authors state that the seedlings, after being smeared with vaseline, "must be placed vertically in the dark for two to seven days to allow the carbon dioxide to accumulate if downward curvature

[of the shoot] is to be demonstrated"; also that "no upward curvature or positive downward curvature in the dark has been observed in unvaselined specimens of maize, *Stellaria media* or sycamore seed-lings." The authors also state that unvaselined shoots in a number of cases have shown downward curvature in the dark; and again, "The treatment with vaseline and darkness also inhibits the growth of the stem." The foregoing statements and quotations from the authors are extraordinary. If their use of vaseline inhibited the growth of the shoots, how then could the shoots later show geotropic curves? To say that the unvaselined shoots of maize and other species in the dark showed no upward curvature is to condemn their own methods; for the testimony of fifty years is against them. Maize is one of the most common of plants whose seedlings are used for laboratory experiment and class demonstration; and, in practice, for the demonstration of geotropic curves, the seedlings are grown in the dark. Everyone who has worked with plant tropisms knows that the shoots of maize and other seedlings turn upward in the dark within an hour or two in usual temperatures.

The last series of experiments was made by Miss Lynn in the attempt to bring reversal of geotropic response in seedling stems by the use of carbon dioxide in closed chambers. After some trials, the author selected *Helianthus annuus* for the tests. She found that a proportion of carbon dioxide of 10 per cent. or over would give reversed curves; of 9 to 10 per cent. would give some positive, some negative; while a percentage of less than 9 would give only negative curves in the shoot. A plate of figures accompanies the text. The figures show that the author's downward bends were not geotropic, but were caused by the sagging of elongated, weak, and etiolated shoots. The most of the sagging shoots in the figures show an upward bend just below the cotyledons, and this is the true geotropic curve— a normal, negatively geotropic curve. A table also accompanies the text, and this shows a higher percentage of carbon dioxide for the so-called reversals in March than in February—a result that may very well be correlated with the relative sun illumination in the two months, if the so-called downward curves were only the sagging of weak stems.

Recently Coupin[2] has published the statement that he found the seedling shoot of the lentil taking the horizontal position when growing in the dark room. Coupin says that the shoot continued in its horizontal position till it died. There have been reports by earlier writers of similar behaviour by other leguminous seedlings; but all these unusual behaviours have been found to be due to the presence of illuminating or other poisonous gas. Coupin makes no mention of precautions against such disturbing influences. If the shoot of

the lentil, or any other seedling, grew always horizontally in the dark, how could one ever raise a crop from planted seed? The buried shoot would never rise out of the ground.

RENEWED ATTEMPTS AT REVERSAL OF GEOTROPIC RESPONSE

In the autumn of 1922 the writer planned a series of experiments designed to re-examine the question of reversal of geotropic response. The most of the experiments were carried through by Miss Anna Haire, a skilful graduate student in the University of Michigan. A detailed narrative of her work will be published in *Scientific Papers of the University of Michigan*, **3**, to appear early in the year 1924. In the present paper only a summary of her work and that of the present writer, so far as it relates to the subject in hand, will be included. All preparations were kept in temperatures from 21 to 25° C.

SERIES I. *With vapour of acetic acid.* In this series an acid atmosphere was given to the shoots of seedlings. The preparations were made in two ways: (1) Seedlings of *Pisum sativum* L. and *Zea mais* L. (yellow dent) were grown in sphagnum moss till the shoots were from 1 to 5 cm. long. The seedlings were then secured to wooden bars by means of blotting paper and rubber bands. The bars with six to twelve seedlings each were then suspended in damp chambers made by lining four-sided glass museum jars with wet absorbent paper. The bars extended across the damp chambers and were wedged securely in place by pieces of rubber tubing. Thirty-three seedlings of *Pisum* and over 200 seedlings of *Zea* were used. Into each damp chamber, after the seedlings had been inserted, were poured 10 c.cm. of 1 per cent. glacial acetic acid, into others the same amount of 5 per cent. into others the same amount of 10 per cent., and into others the same amount of 20 per cent. acid. The jars stood in the dark room with seedlings erect for 5 to 24 hours after receiving the acid. This was done to give the acid time to penetrate more or less into the cells of the seedling shoots. The jars with 1 per cent. and those with 5 per cent. acid received in addition 2 c.cm. of the same strength of acid every 12 hours during the period of the experiment. After standing thus for 5 to 24 hours, the jars were turned on their side, thus bringing the axis of the seedlings into the horizontal position. (2) The other method of preparation was to grow the seedlings in the dark room in earth in crystallising dishes 6 cm. in diameter. When the shoots were 1 to 5 cm. above ground, the crystallising dishes with seedlings undisturbed were wedged into four-sided jars, these jars used as culture chambers, and subsequently treated in every way as the jars in the first set, that is, acid was

poured in of the same strengths, the seedlings kept erect for 5 to 24 hours, and the jars then turned over so as to bring the seedlings into the horizontal position. Of course, it was not necessary to turn the seedlings horizontally for the test; for, if the response were reversed by the acid, the stems would turn from the erect position themselves.

The experiments ran from 24 hours to five days. There was not one reversal of geotropic response. In the chambers with vapour from 1 per cent. and those with vapour from 5 per cent. acid, the upward curvature of shoots was normal for three days in the seedlings suspended in air and those growing in earth. In the experiments continued for more than three days in the weaker vapours, the shoots became long and sagged, but the resulting bends were near the bases of the shoots in *Pisum* and not near the tip as they would have been if geotropic. Many roots lived for two to three days in the vapours from concentrations below 10 per cent., but were killed in one day in the vapour from the 10 and 20 per cent. acid. In the two stronger vapours, both roots and shoots were killed; but most shoots lived long enough to make an upward curvature of 20 to 90°.

SERIES II. *Shoots vaselined.* The work of Small and Rea in smearing the stems of seedlings with vaseline to cause thereby the carbon dioxide to accumulate within, and thereby the stems to become acid was repeated except that in the present case other species were used except for *Zea mais*. However, one may safely assume that the behaviour of most plants will be the same with the vaseline treatment. Eighty seedlings of *Zea mais* were used, 37 of *Avena sativa* L., 56 of *Lupinus albus* L., 32 of *Pisum sativum*, 14 of *Vicia faba* L., 15 of *Cucurbita maxima* Duchesne, and 100 of *Helianthus annuus* L.

Smearing the shoots with vaseline or olive oil is very injurious to the plant, as recognised by Small and Rea, who say that the growth of their plants was thereby inhibited. It is singular that these authors should also say that it was necessary to allow their plants to stand vertically in the dark from two to seven days, after applying the vaseline, before the plants were turned horizontally. In the work of Miss Haire and myself, it was found that the plant shoots very soon became translucent, after applying the vaseline, except in the cases of *Avena* and *Zea*. These shoots, being protected by their wrappings of leaves, were not penetrated so quickly by the oil, and generally made negatively geotropic curves, provided they were turned horizontally within 36 hours of the application of the vaseline. The shoots of the other species, if they were very young—1 to 3 cm. long—showed initial upward curves, but their growth and movement soon ceased. If the shoots were longer—8 to 10 cm.—before applying

the vaseline, growth ceased without any curving, and the shoots, after a day or two, sagged of their own weight. There was never in any of the plants a curve that could be called geotropic.

SERIES III. *In carbon dioxide atmosphere.* The seedlings used in this series were all grown in small crystallising dishes in earth, and the dishes with their seedlings were placed in the experimental chambers when the shoots had grown from 1 to 5 cm. above ground. All preparations were kept in the dark. The culture chambers were belljars of 10 litres capacity with tubulature at the top. The jars stood in large basins holding water, the crystallising dishes on supports above the water. The belljars were graduated, and a graduated scale was fastened to the side. An aspirator connected with the culture chambers enabled one to fill the chambers with water to any desired height. The carbon dioxide was let into the chambers from a reservoir or directly from a generator after washing the gas. The water used in the culture chambers was drawn from a small closed tank which contained carbon dioxide in the same concentration as that to be used in the culture chambers. Thus there could be but small exchange of gases between the water and the gas in the culture chambers. To control the percentage of carbon dioxide rather closely, a small quantity of gas was drawn from the culture chamber both just before and just after an experiment, and the amount of carbon dioxide determined by titration. Usually there was an increase of 2 to 4 per cent. in the concentration of the carbon dioxide during the progress of an experiment, due to the respiration of the seedlings. Concentrations of carbon dioxide from 4 to 75 per cent. were used, the most of the seedlings having 10 to 20 per cent.

One hundred and twenty-five seedlings of *Helianthus annuus* were used, and, besides these, 16 of *Lupinus albus*, 13 of *Pisum sativum*, and enough of *Phaseolus vulgaris* L., *Cucurbita maxima*, *Brassica alba* Boess., *Zea mais* and *Avena sativa* to bring the total to 250 seedlings.

None of the seedlings showed reverse curves. The *Helianthus* shoots made upward curves in all the percentages of carbon dioxide used—from 4 to 20 per cent. *Lupinus* bent its hypocotyl upward in 20 and 50 per cent.; older seedlings of *Phaseolus* bent the epicotyl upward while the mature hypocotyl remained straight; *Pisum* epicotyls bent sharply upward in 12 and 20 per cent.; *Cucurbita* bent slightly upwards in 33 per cent.; *Brassica* did not grow but lost its turgidity and sagged down in 75 per cent.; *Zea* had unfolded its first leaves when placed in an atmosphere of 35 per cent., but made slight upward bends—about the same behaviour as shown by controls.

The plants continued from one to four days in carbon dioxide atmospheres. It frequently happened in the concentrations of 12 to 20 per cent. that the shoots grew long and then, after having made an upward bend, swung laterally by their weight, twisting the axis and so bringing the shoot into a pendent position.

SERIES IV. *With ammonia vapour.* In the attempt to surround the roots with an alkaline atmosphere, ammonia vapour was used—the same means as employed by Small, but by a different method. To start with, one-twelfth normal ammonium hydrate was prepared. From this solution were made 0·5, 0·25, 0·12, 0·06 and 0·03 per cent. of the twelfth normal solution. The seedlings used were those of *Zea mais* and *Vicia faba*. Sixty of *Zea* and 52 of *Vicia* were successfully carried through the tests, after favourable strengths of vapour were determined. The seedling roots were 1 to 3 cm. long when the seedlings were fastened to wooden bars and placed in desiccators made into damp chambers. The tubulature of the desiccator was in the top and was closed with a rubber stopper through which passed a glass tube for the supply of ammonia. The glass tube was, of course, kept closed except when introducing the ammonia. Red litmus paper was suspended in each damp chamber as an indicator. When the seedlings were first suspended in the damp chambers 5 c.cm. of the ammonia solution was inserted, and the same amount was introduced every five or six hours thereafter to the end of the experiment.

In the vapour from the 0·5 per cent. ammonia, the roots did not grow but soon became brown and died. In vapour from 0·25 per cent. the roots grew but a few millimetres, some remaining straight, and some becoming twisted and distorted, bending upward, downward, and in all directions. After 24 hours in this strength of ammonia, the roots were brown and dead. In the chambers with 0·12, 0·06 and 0·03 per cent., the growth of roots was good, and all roots showed positively geotropic curves. The majority of seedlings were kept in vapour from 0·12 per cent. ammonia, as this was the greatest strength in which a fair amount of growth could be made.

It would seem that the conclusion to be drawn from the examination of the work of Small and his associates, and from the results of similar experiments recorded in the foregoing pages, is that there is as yet no evidence for the reversal of geotropic response by such means as those used by those investigators. The conclusions reached by these authors seem to the writer to have been due to misinterpretations of distortion figures and to the behaviour of sagging shoots, too old and too weak to hold themselves erect.

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